An Open-Label, Two-Arm, Phase I Trial of Recombinant Human Interleukin-21 in Patients with Metastatic Melanoma

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Abstract

Purpose: Human interleukin-21 (IL-21) is a pleiotropic class I cytokine that activates $CD8^+$ T cells and natural killer cells. We report a phase 1 study of recombinant human IL-21 in patients with surgically incurable metastatic melanoma. The primary objective was to investigate safety and tolerability by determining dose-limiting toxicity (DLT). The secondary objectives were to identify a dose response for various biomarkers in the peripheral blood, estimate the minimum biologically effective dose, determine the pharmacokinetics of IL-21, determine if anti-IL-21 antibodies were induced during therapy, and measure effects on tumor size according to Response Evaluation Criteria in Solid Tumors.

Experimental Design: Open-label, two-arm, dose escalation trial of IL-21 administered by i.v. bolus injection at dose levels from 1 to 100 μg/kg using two parallel treatment regimens: thrice weekly for 6 weeks (3/wk) or three cycles of daily dosing for 5 days followed by 9 days of rest (5+9).

Results: Twenty-nine patients entered the study. IL-21 was generally well tolerated and no DLTs were observed at the 1, 3, and 10 μg/kg dose levels. In the 3/wk regimen, DLTs were increased in alanine aminotransferase, neutropenia, and lightheadedness with fever and rigors. DLTs in the 5+9 regimen were increased in aspartate aminotransferase and alanine aminotransferase, neutropenia, fatigue, and thrombocytopenia. The maximum tolerated dose was declared to be 30 μg/kg for both regimens. Effects on biomarkers were observed at all dose levels, including increased levels of soluble CD25 and up-regulation of perforin and granzyme B mRNA in $CD8^+$ cells. One partial tumor response observed after treatment with IL-21 for 2 × 6 weeks (3/wk) became complete 3 months later.

Conclusions: IL-21 is biologically active at all dose levels administered and is generally well tolerated, and phase 2 studies have commenced using 30 μg/kg in the 5+9 regimen.

The incidence of melanoma has increased dramatically over the last four decades and melanoma is now one of the most common forms of cancer. In the United States, it was estimated that ~60,000 patients will be diagnosed with melanoma and ~8,100 patients will die from the disease in 2007 (1). The incidence in Australia is twice as high as in the United States at 40/100,000 per year (2). Mortality is in the range 2 to 5/100,000 per year and although this has improved with earlier detection, mortality from metastatic melanoma has not improved. The 5-year survival for metastatic melanoma is 5% to 10% with a median survival of the order of 7 months (3), although a small proportion of patients presenting with metastatic disease can survive for many years (4).

Current approved treatment options for metastatic melanoma include dacarbazine and interleukin (IL)-2. Dacarbazine has shown a response rate of <10% in several studies, with median response duration from 4 to 8 months. Other single-agent chemotherapeutic regimens have not shown improved response rates or response durations. Single-agent therapy with IL-2 has shown similar overall response rates but with a small percentage of durable complete responses (5). Use of this biological agent has been complicated by considerable toxicities (6). Combinations of biological and cytotoxic therapies have not improved outcomes (7). New therapeutic regimens are therefore needed for the management of late-stage melanoma.

IL-21 is a cytokine produced by activated $CD4^+$ T cells as a polypeptide of 131 amino acids with an approximate molecular weight of 15.6 kDa (8). It is a four-helix bundle class I cytokine (9) and has significant homology/identity to IL-2, IL-4, and IL-15. The IL-21 receptor consists of an IL-21–specific α chain

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that heterodimerizes and signals through the common cytokine receptor γ chain (γ_c), shared with other cytokines in this family (IL-2, IL-4, IL-7, IL-9 and IL-15; ref. 10). Signaling via the IL-21 receptor heterodimer involves activation of Janus-activated kinase (JAK) 1, Janus-activated kinase 3, signal transducers and activators of transcription (STAT) 3, and extracellular signal-regulated kinase (ERK) 1/2 (10, 11). Expression of IL-21 receptor is restricted primarily to spleen, thymus, lymph node, and peripheral blood mononuclear cells (8).

IL-21 stimulates IFN-γ production and enhances the cytolytic activity of natural killer (NK) cells and $CD8^+$ T cells in synergy with IL-2 and IL-15 (12). IL-21 potently augments IL-15–induced proliferation of murine $CD8^+$ T cells (13) and promotes clonal expansion of antigen-stimulated human $CD8^+$ T cells (14). IL-21 also has potent effects on B-cell proliferation and antibody production. It enhances B-cell proliferation induced by T-cell–dependent signals and inhibits B-cell proliferation induced by T-cell–independent signals (8). When stimulated with IL-21, B cells up-regulate IgG responses and diminish IgE responses to antigen (15). Collectively, these *in vitro* data suggest the immunomodulatory effects of IL-21 provide a link between innate and adaptive immunity that may augment the strength and duration of cell-mediated and humoral immune responses.

Preclinical efficacy studies using murine IL-21 have shown significant tumor regression in murine models of metastatic melanoma, renal cell carcinoma, fibrosarcoma, pancreatic carcinoma, colon carcinoma, adenocarcinoma, and thymoma (16–22). The mechanism of action is not clear. Depletion studies have shown that the antitumor activity of IL-21 requires $CD8^+$ T cells, NK cells, or both depending on the tumor model, whereas depletion of $CD4^+$ T cells did not affect the tumor growth (16, 17, 19–21, 23). IL-21 can also augment antibody-dependent cellular cytotoxicity mediated by the antibodies trastuzumab and rituximab (24–26). Thus, the antitumor effect of IL-21 probably involves multiple immunologic mechanisms including both innate and adaptive responses.

In nonhuman primates, daily i.v. administration of recombinant human IL-21 (rIL-21) causes dose-dependent effects consistent with immunomodulation. Schedules of dosing in animal studies have included the 5+9 (treatment for 5 days followed by a 9-day rest period) and 3/wk (dosing thrice weekly continuously) regimens. These were chosen because they are similar to schedules commonly used for cytokine therapy with IL-2 or IFN-α.

rIL-21 is being developed by both ZymoGenetics, Inc. and Novo Nordisk A/S. The initial clinical development of IL-21 aims to test the safety and efficacy of IL-21 in patients with malignancies in which responses to immunotherapy have been described previously. We report a phase 1 clinical trial of IL-21 in patients with metastatic melanoma. Interim results of the trial have been presented at various meetings (27–30).

Materials and Methods

IL-21. rIL-21 was provided by Novo Nordisk A/S. The process for production, refolding, and purification of the molecule as well as analytic methods for assessing purity and potency were developed by ZymoGenetics. rIL-21 is expressed in *Escherichia coli* as the NH_2-terminal methionylated form of the molecule. Manufacturing of the product was carried out in the GMP facilities of Avecia Ltd. Each vial contained 0.8 mL IL-21 at a concentration of 10 mg/mL. At dose levels up to 30 μg/kg, the product was diluted to 0.1 or 1 mg/mL using sterile saline for injection (sodium chloride 0.9%, w/v). At higher doses, no dilution was required.

Trial design. Trial NN028-1614 was an open-label dose escalation phase 1 study of IL-21 administered by i.v. bolus injection. The primary objective was to assess the safety and tolerability of IL-21 at various dose levels and dosing regimens by determining dose-limiting toxicity (DLT) and estimating a maximum tolerated dose (MTD) using the U.S. National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. Secondary objectives were to identify a dose response for various biomarkers in the peripheral blood, estimate the minimum biologically effective dose, determine the pharmacokinetics of IL-21, determine if anti-IL-21 antibodies were induced during therapy, and measure effects on tumor size (if applicable) according to Response Evaluation Criteria in Solid Tumors (31). The patients were allocated nonrandomly in cohorts of two patients into one of two parallel treatment arms with dosing in the morning: treatment thrice weekly (Monday, Wednesday, and Friday) for 6 weeks (3/wk) or three cycles of daily dosing for 5 days followed by 9 days of rest (5+9). The lowest dose level corresponded to 1/10 of the no observed adverse effect level in toxicologic studies with cynomolgus monkeys using different treatment schedules. No modification of doses was allowed for individual patients. DLT was defined as adverse events of grade 3 or higher and at least possibly related to study drug, with exceptions for hyperglycemia, hyperuricemia, and lymphopenia. Laboratory abnormalities were only reported as adverse events if they were at least CTCAE grade 3. The MTD was defined according to protocol as the highest dose at which zero or one patient of five had a DLT. Participants who did not complete week 7 and did not experience DLT were replaced, whereas participants withdrawn due to DLT were not replaced. Following completion of dosing at each dose level, all safety data (adverse events, data of hematology, biochemistry, urinalysis, and vital signs, including ECG and QTc), as well as available pharmacokinetic data and available results from the ongoing analysis of biomarkers, were used by the Study Dose Evaluation Group in the safety evaluation and decision on the next dose level to investigate. The trial schema is shown in Fig. 1.

Patient population. Eligible patients had histologically confirmed surgically incurable metastatic malignant melanoma. Other inclusion criteria include the following: age ≥ 18 years; Eastern Cooperative Oncology Group performance status of 0 or 1; expected life expectancy ≥ 4 months; adequate bone marrow function [WBC ≥ 2.5×10^9/L, absolute neutrophil count ≥ 1.5×10^9/L, platelet count ≥ 100×10^9/L, hemoglobin ≥ 100 g/L, lymphocytes ≥ 1.0×10^9/L (later amended to 0.8×10^9/L), and no sign of hemolytic anemia]; bilirubin ≤ 1.25 × upper limit of normal; aspartate aminotransferase (AST) ≤ 2.5 × upper limit of normal (unless attributable to liver metastases in which case

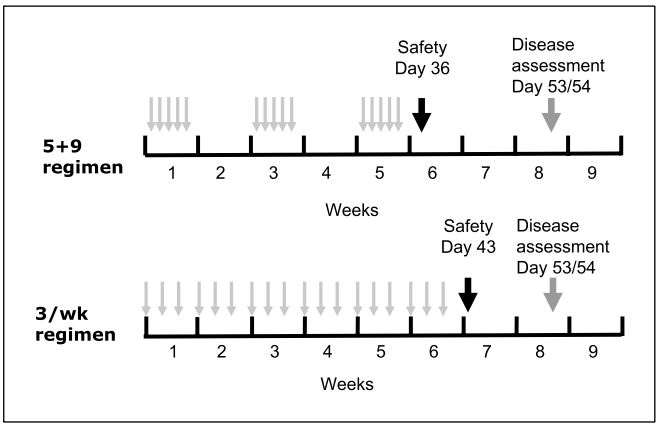

Fig. 1. Study design with two dosing regimens in parallel, the 5+9 regimen and the 3/wk regimen.

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Table 1. Adverse events

	Dose level (μg/kg)						Total
	1	3	10	30	50	100	
No. exposed patients	5 (3)	4 (2)	5 (2)	9 (4)	4 (2)	2 (1)	29 (14)
Fatigue	1 (1)	—	4	7	3 (1)	1 (1)	16 (3)
Pyrexia	2	—	2 (1)	6	4	1	15 (1)
Nausea	3	2	2	4 (1)	2	2	15 (1)
Headache	3	1	2	5	1	1	13
Rash	—	2	1	3	2	1	9
Myalgia	1	—	2	6	—	—	9
Vomiting	1	1	1	3	2	1	9
Pruritus	1	1	1	5	—	—	8
Anorexia	—	1	—	3 (1)	2	2	8 (1)
Influenza-like illness	2	—	1	3	2	—	8
Diarrhea	1	2	2	1	—	1	7
Chills	1	—	2	3	1	—	7
Toxicity							
Lymphopenia	(1)		(1)	(4)	(1)		(7)
Neutropenia				(2)	(1)		(3)
Leukopenia			(1)	(1)			(2)
Anemia			(1)		(1)		(2)
Thrombocytopenia						(2)	(2)
Increased GGT	(1)	(1)	(1)		(2)		(5)
Increased ALT				(2)		(2)	(4)
Increased AST		(1)				(2)	(3)
Hypofibrinogenemia	(1)	(1)		(1)			(3)
Hyponatremia					(1)	(1)	(2)
Hyperkalemia		(1)		(1)			(2)
Increased ALP		(1)					(1)
Hyperbilirubinia		(1)					(1)
Hyperuricemia				(1)			(1)

NOTE: Number of patients with most frequently reported adverse events across the dose levels and with the number of patients with CTCAE severity grade 3 or higher is in parenthesis. Laboratory abnormalities were only reported as adverse events if they were CTCAE grade 3 or higher and therefore given in parenthesis.
Abbreviations: GGT, γ-glutamyl transpeptidase; ALP, alkalinephosphate.

≤ 5 × upper limit of normal); LDH ≤ 2 × upper limit of normal; and calculated creatinine clearance ≥ 60 mL/min. Key exclusion criteria include the following: acute or active infection requiring systemic treatment; hepatitis B/C or HIV positive; autoimmune disease (vitiligo and treated pernicious anemia were permitted); uncontrolled brain metastases or edema; recent chemotherapy, radiotherapy, or major surgery; concurrent systemic corticosteroids (topical or inhalational corticosteroids were permitted); and other prior malignancy (except basal cell or squamous cell skin cancer, carcinoma *in situ* of the cervix, or any curatively treated nonhematologic malignancy with no evidence of recurrence for at least 3 years). Minor surgery for isolated metastases was permitted at the discretion of the principal investigator and such patients were not necessarily removed from the trial.

After the first dose of IL-21, the patients were observed in hospital for 24 to 28 h. Subsequent IL-21 doses were administered in an outpatient setting followed by observation for 4 h including assessments.

Disease assessment was done before treatment and in week 8 according to Response Evaluation Criteria in Solid Tumors (31), with confirmation of responses by repeat imaging at least 4 weeks later. If there was no symptomatic tumor progression at the week 8 assessment that required treatment by another modality, further treatment with use of the same dose and regimen was offered including disease assessment in week 16. At the second evaluation after start of trial, only participants with >30% reduction of tumor size compared with the baseline (before start of treatment) were offered further treatment.

Participants who completed all evaluations up to and including those scheduled for day 5 were evaluable for immunologic end points. Participants who completed at least the first 24-h pharmacokinetic sampling period were evaluable for pharmacokinetic analysis. Participants who completed the second planned tumor evaluation (days 53-54) were evaluable for tumor response. All participants were accounted for in the safety and final analyses.

The study was done between September 2004 and January 2006. All patients were treated at the Austin Hospital, the Peter MacCallum Cancer Centre, or the Royal Melbourne Hospital in Melbourne, Australia, and all patients provided written informed consent before any study-specific procedures. The trial protocol was approved by the Human Research Ethics Committees of Austin Hospital, the Peter MacCallum Cancer Centre, and Royal Melbourne Hospital and was done under the Australian Therapeutic Goods Administration Clinical Trials Notification scheme. The study sponsor was Novo Nordisk A/S. As this was a phase 1 trial, it was not required to be registered with an International Committee of Medical Journal Editors compliant trials registry.

All clinical laboratory tests (hematology, biochemistry, and urinalysis) were done by Mayne Health Dorevitch Pathology. Syne Qua Non Ltd. provided the electronic data capture system. The Cancer Trials Australia Laboratory was responsible for some biomarker sampling/assays. Other assays, including soluble CD25 (sCD25) and mRNA for granzyme B and perforin, were done by Novo Nordisk A/S.

Pharmacokinetics. Blood samples were obtained using venipuncture immediately before dosing and 5, 15, and 30 min and 1, 2, 4, 8, 12, and 24 h after first and second last doses. Serum samples were stored at -70°C until analysis. The IL-21 assay, a sandwich ELISA, was used based on two anti-IL-21 monoclonal antibodies (mAb) produced by Zymogenetics and Novo Nordisk A/S, respectively. The assay has a

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calibration range from 0.25 to 50 ng/mL with a lower limit of quantification of 0.25 ng/mL. Pharmacokinetic end points were calculated by use of noncompartmental analysis: the terminal half life as $t_{1/2} = \ln(2) / \lambda_z$, where λ_z is the terminal rate constant and determined as the slope of the linear regression line in a logarithm to the concentration-time plot; $AUC_{0\text{-inf}}$ as area under the curve (AUC) = $AUC_{0\text{-t}} + C_t / \lambda_z$, where C_t is the last quantifiable concentration; $AUC_{0\text{-t}}$ using the trapezoidal rule; CL as CL = Dose / AUC after single dose and CL = Dose / $AUC_{0\text{-}\tau}$ at steady state; V_z as V_z = CL / λ_z; and V_{ss} as V_{ss} = CL ×·mean residence time. The calculations were done by use of SAS for Unix, version 8.02.

All analyses for safety, pharmacokinetic, and pharmacodynamic end points were done using all available data, unless otherwise stated. Missing values were not imputed.

Soluble CD25. Serum samples were obtained as described above and stored at -70°C until analysis. sCD25 was quantified using a sandwich ELISA (Quantikine, DR2A00, R&D Systems). In brief, the assay captures sCD25 in 25% human serum with a mAb coated onto microtiter plates. Captured sCD25 was revealed using an enzyme labeled polyclonal antibody specific for sCD25. The assay has a calibration range from 78 to 5,000 pg/mL.

Quantitative real-time PCR. Citrated blood samples were collected from patients on day 1 (before first IL-21 dose) and on day 5. $CD8^+$ cells were enriched using CD8 Microbeads (Miltenyi Biotec) and a magnetic MiniMACS Separation Unit (Miltenyi Biotec). $CD8^+$ fractions were lysed in RNA extraction buffer (Zymo Research). Total RNA was isolated using Mini RNA Isolation I kit (Zymo Research). RNA integrity was confirmed using the Agilent 2100 Bioanalyzer and total RNA Nano chips (Agilent Technologies). cDNA was prepared using random primers and Taqman Reverse Transcription reagents (Applied Biosystems). Quantitative real-time PCR was done using Taqman PCR core reagents and the ABI Prism 7900HT System (Applied Biosystems). Primers and FAM-labeled probes for perforin-1 mRNA, granzyme B mRNA, and 18S rRNA were ordered as Assays-on-Demand (Applied Biosystems).

Immunogenicity. Antibodies against IL-21 were measured by ELISA. In brief, ELISA plates were coated overnight with 0.25 μg/mL IL-21 and samples (human serum), QC samples (rabbit anti-IL-21), and negative samples (human 0 samples) were applied and incubated 1 h at room temperature. Detection reagents were peroxidase-labeled donkey anti-human IgG and donkey anti-rabbit IgG (Jackson ImmunoResearch). Assay cutoff was estimated from a panel of normal samples as mean + two SDs. Samples above cutoff were confirmed by inhibition with surplus of IL-21.

Results

Patients. Twenty-nine patients were entered into the trial, consisting of 13 females and 16 males with a median age of 59 years (range, 28-74); Eastern Cooperative Oncology Group performance status of 0 to 1 (22 and 7 patients, respectively); median number of target lesions three (range, 1-6); median sum of target lesions 7.7 cm (range, 1.5-17.5); median body weight of 75 kg (range, 56-109); and all were Caucasians. Although formal subclassification into M1a, M1b, or M1c according to American Joint Committee on Cancer was not recorded before study entry, a retrospective classification based on baseline information indicated that for 26 evaluable patients, 5 patients were M1a, 6 were M1b, and 15 were M1c. One patient was found to be ineligible (lymphopenia) after entering the study and was withdrawn. Twenty-one patients completed the main study with 6 weeks of IL-21 treatment, 10 patients received further treatment with IL-21 for 6 weeks and one of those patients completed a further 6 weeks giving a total of 18 weeks of dosing at the 30 μg/kg dose level using the 3/wk regimen for that patient.

Safety and toxicity. Adverse events are described in Table 1. All 29 patients experienced at least one adverse event, most of which were thought to be related to IL-21 and which were mild or moderate in severity. The most common adverse events were fatigue, fever, nausea, and headache, occurring in ~50% of patients. Other common adverse events included influenza-like symptoms, chills, vomiting, rash, pruritus, myalgia, and anorexia, overall occurring in ~25% of patients. There were no clear differences in the frequency of these common adverse events between the two dosing regimens and across dose levels.

No DLTs were observed at the 1, 3, and 10 μg/kg dose levels. In the 3/wk regimen at the 30 μg/kg dose level, three of seven patients experienced DLTs: increased alanine aminotransferase (ALT) on day 31 (325 units/L, grade 3); neutropenia (brief neutropenia, 0.7×10^9/L grade 3 but not considered clinically significant); and lightheadedness in conjunction with grade 2 fever and rigors in a patient with previous brain metastases, respectively. After detailed discussion of the clinical significance of the three cases with DLTs according to the CTCAE in the group of seven patients, the Study Dose Evaluation Group determined that a dose <30 μg/kg could not be declared as the MTD but also determined that it would not be safe to increase the dose further. Thirty micrograms/kilogram were therefore declared the MTD for the 3/wk regimen. In the 5+9 regimen, dose escalation continued to the 100 μg/kg dose level before DLTs were observed. At the

Table 2. Results of pharmacokinetic analysis after the first dose of IL-21

	Dose level (μg/kg)					
	1	**3**	**10**	**30**	**50**	**100**
n	4	4	4	9	4	2
$AUC_{0\text{-inf}}$ C (ng·h/mL; $AUC_{\%extrap}$)	2.9 (26.1)	19.9 (10.8)	66.0 (1.8)	193 (0.9)	336 (1.2)	476 (1.4)
C_{5min} (ng/mL)	1.8	9.7	32.8	107	194	270
$t_{1/2}$ (h)	1.1	1.3	1.7	3.0	3.3	4.2
CL (mL/kg/h)	339	151	152	155	149	210
V_{ss} (mL/kg)	523	262	344	437	533	949
V_z (mL/kg)	529	277	376	666	713	1287

NOTE: Geometric means: $AUC_{0\text{-inf}}$ = AUC until infinity; $AUC_{\%extrap}$ = area extrapolated after last quantifiable concentration; C_{5min} = IL-21 concentration 5 min after injection; $t_{1/2}$ = terminal half-life; CL = total clearance of IL-21 from plasma; V_{ss} = apparent volume of distribution at equilibrium; V_z = apparent volume of distribution during the terminal phase.

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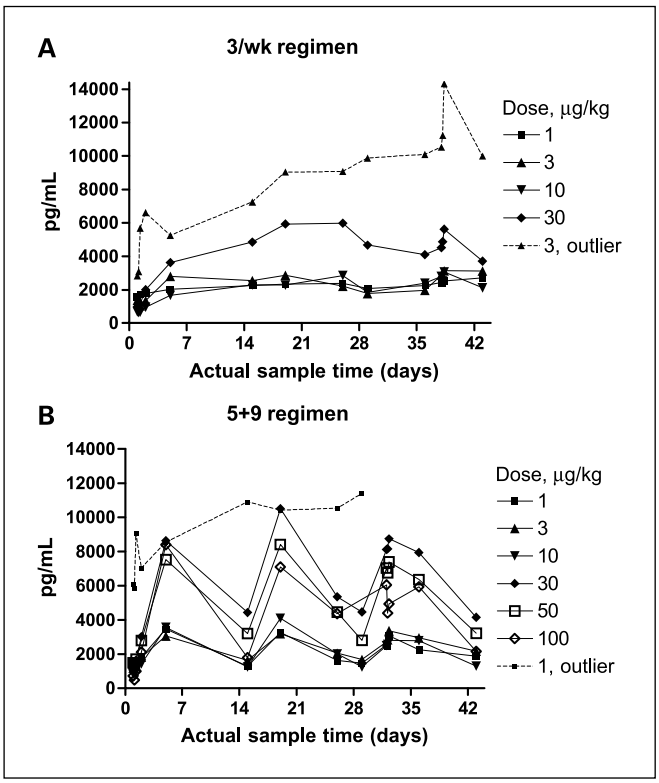

Fig. 2. Serum levels of sCD25. sCD25 was measured in serum samples at the indicated time points by ELISA. *A,* 3/wk regimen. *B,* 5+9 regimen. The mean of each dose level is shown for each dosing regimen. Two patients with outlying sCD25 values (defined as greater than mean + three SDs at day 1) have been omitted from the mean calculations but are shown with the broken lines. These two patients have several other outlying values, including neutrophils and WBC counts (data not shown).

100 μg/kg dose level, one patient had grade 4 thrombocytopenia (23×10^9/L), together with grade 3 increase of AST and ALT and grade 3 fatigue. Because DLTs had already been observed in several patients at 30 μg/kg in the 3/wk dose regimen, for safety reasons, two more patients were added at a lower dose level (50 μg/kg) instead of enrolling three more patients at 100 μg/kg according to the protocol. At the 50 μg/kg dose level, one of four patients experienced DLT (grade 3 neutropenia and grade 3 fatigue). Thus, although higher doses were tolerated by some patients, 30 μg/kg were also declared the MTD for the 5+9 regimen, and this dose was chosen for further phase 2 studies.

A total of 30 serious adverse events were reported in 13 of the 29 exposed patients. Only five (increased ALT, fever, fatigue, nausea, and neutropenia) occurred in more than one patient. One death occurred during the study. The patient had a history of ischemic heart disease, hypertension, and abdominal aortic aneurysm. The patient was admitted to hospital with acute myocardial infarction and died. The death was not thought to be related to treatment with IL-21.

Pharmacokinetics. Twenty-seven patients were included in the pharmacokinetic assessments. The pharmacokinetics of IL-21 showed a rapid multiexponential decline of IL-21 in serum with $t_{1/2}$ between 1.1 and 4.2 h at all dose levels (Table 2). Exposure of IL-21, as assessed by $AUC_{0\text{-inf}}$ and C_{5min}, increased in a dose-dependent manner, indicating linear pharmacokinetics. Generally, there was an increase in CL and thus a decrease in AUC after multiple dosing of rIL-21. After the first dose and second last dose, the serum $t_{1/2}$ ranged between 1.1 to 4.2 h and 0.9 to 3.8 h, respectively. The $t_{1/2}$ increased with increasing dose, which could be explained by the IL-21 concentrations in the terminal elimination phase being below the lower limit of quantification for the lower doses. This is also reflected in the calculation of CL, V_z, and V_{ss}. No accumulation of IL-21 was observed in serum and no major difference in pharmacokinetics between the two dose regimens was observed.

Biomarkers. sCD25 (IL-2Rα) is cleaved from T and NK cells on activation (32). We measured the serum level of sCD25 at multiple time points throughout the trial as a marker of immune activation. In the 5+9 regimen, sCD25 consistently increased during dosing and subsequently returned to baseline during resting periods in all subjects except one with an abnormally high predose sCD25 level (greater than population mean + three SDs). This patient also had other abnormal values

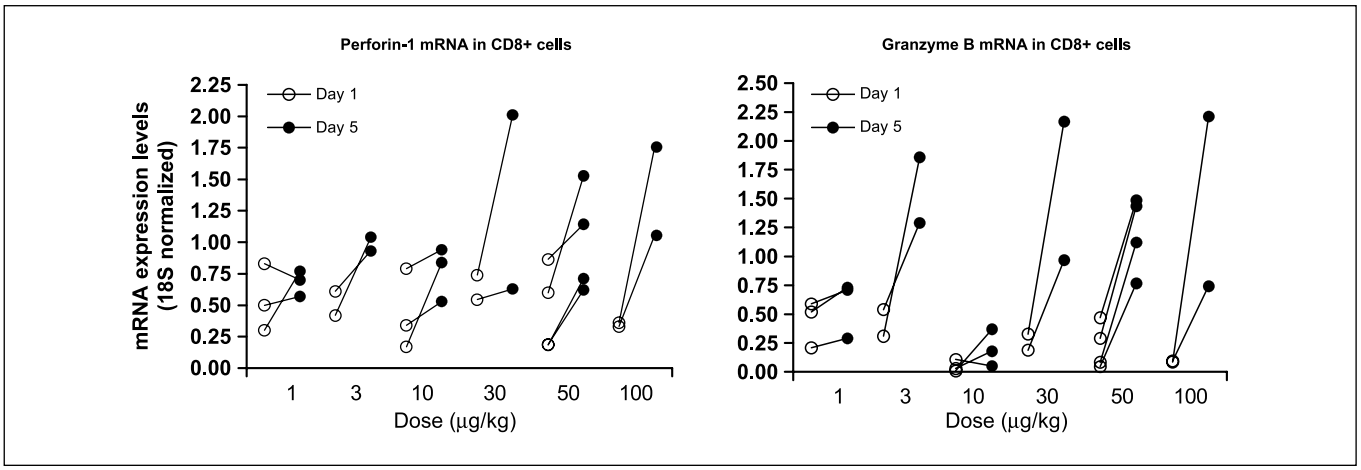

Fig. 3. Effect of IL-21 dosing on perforin-1 and granzyme-B mRNA levels in $CD8^+$ cells. Comparisons of day 1 (predose) and day 5 samples in individual patients from the 5+9 regimen. $CD8^+$ cells were isolated by MACS purification from peripheral blood mononuclear cell, and the mRNA expression levels of the perforin-1 and granzyme B genes were determined by quantitative real-time PCR. The expression levels were normalized to 18S levels. $P < 0.0005$, for both perforin-1 and granzyme B in paired Student's t test.

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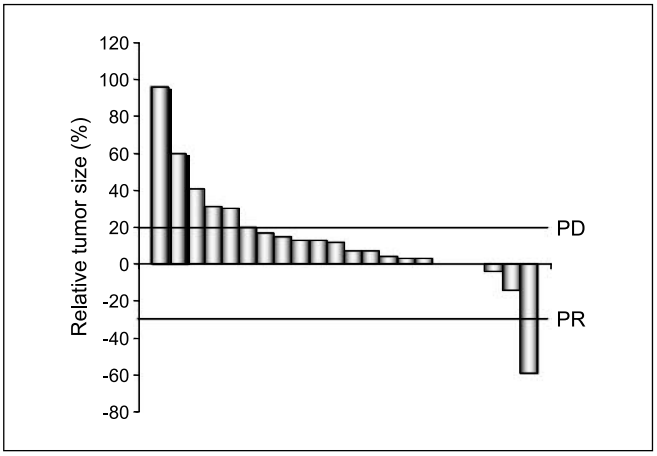

Fig. 4. Waterfall plot showing tumor response of target lesions for 23 evaluable patients at 8 or 16 weeks after initiation of treatment with IL-21 (corresponding to one or two 6-wk treatment cycles of IL-21 dosing plus 2 weeks of follow-up). PD, progressive disease; PR, partial response.

including neutrophilia (data not shown) and was the patient who subsequently died of myocardial infarction during the trial. By contrast, sCD25 increased initially during the 3/wk regimen and subsequently maintained elevated levels during the 6-week treatment (Fig. 2). Dose-dependent increases in sCD25 were observed in both regimens with markedly higher increases in sCD25 at dose levels of 30 μg/kg and above compared with the lower dose levels ($P < 0.002$ and $P < 0.02$ for 5+9 and 3/wk regimens, respectively, comparing day 5 levels by Student's t test).

Perforin and granzyme B are cytotoxic proteins that are released from activated NK cells and $CD8^+$ T cells and induce apoptosis in target cells. These effector proteins are up-regulated on activation of $CD8^+$ T and NK cells and indicate acquisition of cytolytic potential. Perforin-1 and granzyme B mRNA expression levels in purified blood $CD8^+$ T cells generally increased in the patients following IL-21 treatment (Fig. 3). Both perforin-1 and granzyme B mRNA were significantly up-regulated on day 5 compared with day 1 predosing level ($P < 0.0005$, paired Student's t test). The increase was most marked for granzyme B mRNA in patients dosed with 30, 50, and 100 μg/kg, with an approximate 10-fold up-regulation of granzyme B mRNA. A similar trend was observed in NK cells, although the magnitude of the increase was less (data not shown). This trend correlated with increases in NK cytolytic activity in five of six evaluable patients, although absolute NK numbers in peripheral blood decreased (data not shown). These results suggest that effector functions of $CD8^+$ T and NK cells may be increased following IL-21 treatment.

Immunogenicity. Anti-IL-21 antibodies were not detectable in any sample.

Tumor response. Nine patients were assessed as stable disease and one patient on the 3/wk regimen at the 30 μg/kg dose level achieved a partial response (Fig. 4). All 10 patients went on to receive further treatment. The patient with the partial response had participated previously in another clinical immunotherapy trial (33), before relapsing and entering the current study. The patient eventually completed three treatment periods of 6 weeks before withdrawing from the study. A small residual mass was eventually resected 3 months after completing the study and was shown to contain no residual viable tumor. Fourteen months later at the most recent evaluation, the patient was still in complete remission without any sign of malignancies. Fifteen patients had progressive disease and were withdrawn after IL-21 treatment for 6 weeks.

Discussion

The study has shown that IL-21 is generally safe at doses up to 30 μg/kg when administered in either the 5+9 (once daily dosing for 5 days followed by 9 days of rest) or 3/wk (thrice weekly) regimen to patients with metastatic melanoma, although higher doses (50 and 100 μg/kg) were administered and well tolerated by some patients in the 5+9 regimen. A dose of 30 μg/kg was declared to be the MTD and has been incorporated into subsequent phase 2 study designs.

Adverse events were somewhat similar in nature to those observed with other cytokines. However, severe toxicities, such as capillary leak syndrome or severe neuropsychiatric toxicity associated with IL-2 or IFN-α (6), were not observed. DLTs were primarily neutropenia, thrombocytopenia, fatigue, and increased AST and ALT.

The pharmacokinetics of IL-21 indicate rapid clearance from the blood. IL-21 induced effects on a variety of biomarkers, including induction of phosphorylation of intracellular STAT3 *in vivo* within 15 min of injection even at the lowest dose level tested (data not shown). Up-regulation of perforin and granzyme B mRNA expression in $CD8^+$ cells and increased sCD25 in serum was observed, suggesting that IL-21 induced immune activation and augmented the cytolytic potential of these cells. Serum sCD25 increased in both regimens and exhibited a cyclical response in the 5+9 regimen, whereas, in the 3/wk regimen, sCD25 increased initially to reach a more constant level, reflecting the more continuous treatment in this regimen. Even after several weeks of treatment, acute increases in sCD25 were observed soon after dosing (Fig. 2), suggesting that tachyphylaxis of this response to IL-21 did not occur. No clear differences between the two treatment schedules were apparent with respect to toxicity or biological effects.

Although clinical antitumor effects were not a primary end point, several patients had stable disease and one confirmed partial response was observed. In view of this, we have commenced a phase 2 study of IL-21 in patients with metastatic melanoma. Responses have also been observed in patients with metastatic renal cell carcinoma (29), suggesting that IL-21 may have significant antitumor activity as a single agent. Other treatment regimens are being tested, including an ongoing phase 1 study of s.c. dosing (Eudract no. 2006-000376-32). Preclinical studies suggest that IL-21 enhances antibody-dependent cellular cytotoxicity (24–26). Future clinical trials will investigate the combination of IL-21 with rituximab in $CD20^+$ B-cell non-Hodgkin's lymphoma, or cetuximab in patients with colorectal cancer (Eudract No. 2006-004231-30), or combinations with active vaccination approaches.

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