Administration of R24 Monoclonal Antibody and Low-Dose Interleukin 2 for Malignant Melanoma[1]

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ABSTRACT

R24 is a monoclonal antibody that recognizes the disialoganglioside G_{D3} expressed on the surface of malignant melanoma cells. Once bound, it can mediate destruction of these cells through both complement-mediated lysis and antibody-dependent cellular cytotoxicity. Agents such as interleukin 2 (IL-2), which can augment effector cell function and promote destruction of antibody-coated tumor cells, might produce improved antitumor responses when combined with R24. In this series, we evaluated the combination of R24 and IL-2 in a Phase 1b study in patients with metastatic melanoma. Twenty-eight patients with metastatic melanoma were entered into the protocol at two institutions. Patients received 8 weeks of IL-2 by continuous i.v. infusion at a dose (4.5×10^5 Amgen units/m^2/day) designed to selectively expand natural killer (NK) cells. In weeks 5 and 6, patients received R24 for a total of four doses. Twenty-four h after each R24 infusion, patients received a 2-h bolus dose of IL-2 to help promote activity of NK effectors against antibody-coated melanoma targets. Additional IL-2 boluses were administered in weeks 7 and 8. Doses were escalated through two bolus doses of R24 (5 or 15 mg/m^2) and two bolus doses of IL-2 (2.5 or 5.0×10^5 units/m^2). Although one patient experienced severe capillary leak syndrome during IL-2, therapy was otherwise well tolerated. At the higher dose level of R24, two of four patients experienced transient but severe abdominal and chest discomfort, necessitating dose reduction. One patient with ocular melanoma and liver metastases had a partial response. Two additional patients had minor responses. A dramatic increase in NK cell number was noted as a result of treatment, as was augmentation of cytolytic activity against cultured NK-sensitive targets. Antibody-dependent cellular cytotoxicity against cultured melanoma cells in the presence of exogenous R24 or in the presence of serum obtained from patients following R24 infusion also increased during treatment. Our experience indicates that R24 and low-dose IL-2 can be safely combined in patients with metastatic melanoma and that this combination can promote destruction of cultured melanoma cells. The clinical activity of this combination against ocular melanoma may merit further investigation.

INTRODUCTION

Immunotherapeutic approaches to the treatment of malignant disease have held considerable appeal for investigators over the last several decades. The introduction of hybridoma technology permitted the production of large numbers of antibodies directed at a variety of antigenic moieties on tumor cells (1). Labeling studies revealed that many of these antibodies localized to sites of tumor metastases. It was hoped that the therapeutic use of these antibodies would allow tumors to be specifically targeted without injuring normal nonmalignant tissues, and a number of trials of serotherapy have been conducted in patients with metastatic cancer (2–5). Although clinically significant tumor regressions have been observed in several instances, dramatic and sustained responses are rare occurrences.

One disease for which serotherapy trials have been conducted is malignant melanoma. R24, a murine IgG3 monoclonal antibody that recognizes the disialoganglioside G_{D3}, has been one of the most intensely studied. R24 can detect G_{D3} at low density on normal human melanocytes as well as on astrocytes, pancreatic islet cells, adrenal medullary cells, and 15–25% of peripheral blood T cells (6–10). Because G_{D3} is expressed in particularly high concentration on the plasma membrane of malignant melanoma cells, R24 has been used in clinical trials of patients with metastatic disease, and tumor regressions have been observed (11–14).

In cultured melanoma cells, R24 has been demonstrated to mediate ADCC[3] and complement fixation, and it has been postulated that R24 may act *in vivo* by binding directly to melanoma cells, stimulating clearance by immune effector cells through Fc receptor interactions (15–17). Potentiation of the activity of these effector cells might augment the antitumor efficacy. ADCC is mediated, in part, by NK cells, and the capacity of NK cells to destroy antibody-coated cultured tumor targets is enhanced *in vitro* by preincubation of these effectors

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[3] The abbreviations used are: ADCC, antibody-dependent cellular cytotoxicity; IL-2, interleukin 2; IL-2R, IL-2 receptor; NK, natural killer; PBMC, peripheral blood mononuclear cell; ICAM, intercellular adhesion molecule.

with IL-2 (18, 19). We have demonstrated previously that very low doses of IL-2 delivered by continuous infusion to patients with metastatic cancer selectively expands and activates NK cells, with little or no effect on T lymphocytes (20, 21). These low doses of IL-2 are well tolerated, and treatment can be sustained in the outpatient setting for prolonged periods. Moreover, exposure of NK cells from patients receiving low-dose IL-2 *in vivo* to additional IL-2 *in vitro* can dramatically increase cytolytic activity and ADCC against tumor targets (19–21). In a recently completed clinical trial, we demonstrated that relatively low bolus doses of IL-2 *in vivo* could activate primed NK cell populations previously expanded in patients by prolonged continuous infusions of low dose IL-2 (22).

Based upon these observations, we designed a Phase 1b clinical study in patients with metastatic melanoma, combining R24 administration with boluses of IL-2 in patients receiving continuous i.v. infusions of low-dose IL-2. It was postulated that a large activated population of NK cells might aid in the destruction of melanoma cells bound by R24. We find that this approach is clinically tolerable and provides a basis for additional studies of monoclonal antibody therapy and selective NK cell manipulation in patients with malignant disease.

PATIENTS AND METHODS

Patient Eligibility. Patients were eligible for this study if they had evidence of metastatic melanoma demonstrable either by physical exam or radiographic imaging. Patients with either cutaneous or ocular melanoma were candidates for therapy. Patients with only locoregional nodal recurrences were excluded, as were patients with a history of central nervous system metastases. Eligibility criteria included age >18 years, Eastern Cooperative Oncology Group performance status 0–2, normal or near normal parameters of renal, hepatic, and pulmonary function, and life expectancy exceeding 3 months. The trial was conducted at two institutions (Dana-Farber Cancer Institute and Memorial Sloan-Kettering Cancer Institute). Twenty-eight patients (23 from the Dana-Faber Cancer Institute and 5 the Memorial Sloan-Kettering Institute) were registered between October 1992 and March 1995. All patients signed informed consent. The protocol was approved by the institutional review boards of both institutions.

Treatment Design. The schema outlining the planned treatment protocol is depicted in Fig. 1. Patients received recombinant IL-2 (Amgen, Inc., Thousand Oaks, CA) by continuous i.v. infusion through an indwelling central catheter for 8 consecutive weeks at a scheduled dose of 4.5×10^5 Amgen units/m^2/day. One Amgen Unit is approximately the equivalent of 1.8 IU. Drug was delivered by a portable computerized ambulatory pump. The supply of IL-2 was renewed every week in the outpatient clinic. In both the fifth and sixth week of continuous infusion IL-2, patients received two doses of R24 (Lonza Biologic, Inc., Portsmouth, NH) at 5 or 15 mg/m^2 (four doses total) over 2–3 h. These doses had previously been found to be clinically tolerable in patients receiving R24 alone (11, 12, 14). Patients were generally premedicated with diphenhydramine, acetaminophen, and cimetidine before R24 infusion. Twenty-four h following completion of each R24 infusion, patients were given additional 2-h boluses of IL-2 at doses of

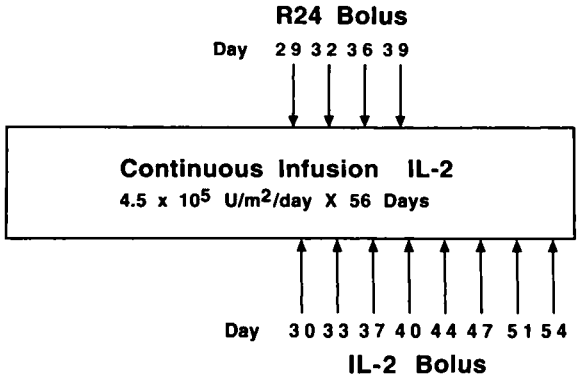

Fig. 1 Treatment schema of IL-2/R24 administration to patients with metastatic melanoma. Two dose levels of R24 (5 or 15 μg/m^2) and bolus IL-2 (2.5 or 5.0×10^5 units/m^2) were evaluated.

$2.5–5.0 \times 10^5$ units/m^2. These bolus doses of IL-2, although very low, had been found previously to activate NK cells expanded *in vivo* by low-dose continuous infusion IL-2 (22). Additional boluses of IL-2 were administered in the seventh and eighth week while patients continued to receive continuous infusion IL-2. All boluses were administered in the outpatient clinic. Cohorts of three to five patients were initially registered for treatment at each dose level. Doses were escalated through two bolus doses of R24 (5 or 15 mg/m^2) and two bolus doses of IL-2 (2.5 or 5.0×10^5 U/m^2) until dose-limiting toxicity was encountered. The first cohort was treated with continuous infusion IL-2 and R24 boluses alone without the additional IL-2 boluses during weeks 5–8. Patients were not routinely treated with any prophylactic antipyretic or anti-inflammatory agents while receiving continuous infusion IL-2. However, they were given acetaminophen and antihistamines prior to the administration of IL-2 boluses and R24.

Clinical Assessment. Tumor measurements were taken within 2 weeks before and after the 8-week treatment program. A complete response was defined by total regression at all tumor sites. A partial response was defined by greater than a 50% reduction in the size of tumor masses, as measured by the bidimensional product of the horizontal and vertical dimensions. A minor response was defined by a 25–50% reduction in the size of tumor masses. Stable disease was defined as less than a 25% shrinkage or growth of tumor masses. Progressive disease was defined as greater than a 25% growth of tumor size or any new sites of tumor developing after treatment was begun. Side effects were assessed by the WHO clinical toxicity criteria.

Immunophenotypic Studies. PBMCs for immunological studies were obtained weekly. During weeks that patients received R24 and IL-2 boluses, samples were obtained prior to the bolus, immediately after completion (0 h), 3 h after completion, and 24 h after completion. Blood was collected in preservative-free heparin. PBMCs were obtained following Ficoll-Hypaque density gradient sedimentation. PBMCs were analyzed by direct immunofluorescence for reactivity with a series of monoclonal antibodies using standard techniques. Cells were analyzed for reactivity with a panel of monoclonal antibodies, including T3 (CD3), T4 (CD4), T8 (CD8), NKH1

(CD56), IL-2R α chain (CD25), IL-2R β chain, Mol (CD11b), and ICAM (CD54; Coulter Immunology, Hialeah, FL). Single and dual color immunofluorescence reactivity was determined by automated flow cytometry analyzing 10^4 cells in each sample (ELITE; Coulter Electronics, Hialeah, FL).

Cytotoxicity Assays. Cryopreserved PBMCs were thawed and evaluated for their ability to lyse NK-sensitive targets (K562). After thawing, effector cells were incubated for 18 h in media alone (RPMI 1640 with 10% heat-inactivated human AB serum, 2% glutamine, 1% penicillin-streptomycin, and 1% sodium pyruvate) or media enriched with IL-2 (500 units/ml, 3 nM; Amgen, Inc.). Four-h chromium release assays against K562 were performed at E:T ratios of 20:1 and 10:1, as described previously (21). Assays were performed on cells prior to beginning IL-2 therapy, while receiving continuous infusion IL-2 prior to R24 bolus, and immediately after R24 bolus.

For ADCC assays against melanoma tumor targets, SK-MEL-28 cells were preincubated in media or in R24 at 100 μg/ml for 1 h and then labeled with ^{51}Cr for 1 h at 37°C. Four-h chromium release assays were performed as above with PBMC effectors obtained from patients prior to initiating treatment and PBMCs obtained during week 5 of therapy with IL-2. Effectors were incubated for 18 h with either media alone or media enriched with IL-2 (500 units/ml) prior to plating cells with targets (5000 cells/well) at E:T ratio of 20:1 or 10:1. Cytotoxicity assays were also performed against SK-MEL-28 in the presence of serum obtained from patients receiving continuous infusion IL-2 immediately before and after R24 bolus to determine whether antibody infusions resulted in concentrations sufficient to promote cytotoxic destruction of melanoma targets. SK-MEL-28 targets were incubated for 1 h in media alone or in serum obtained from patients before or after R24 bolus. Effectors in this assay were PBMCs obtained from patients during the fifth week of therapy. Chromium release assays were performed as above.

Statistical Considerations. Descriptive statistics are reported as proportions, and medians and means are reported with SEs. Cytotoxicity and ADCC assays were all performed in triplicate, and the mean value was used for comparisons between time points. Paired *t* tests were used to compare changes in immune phenotype over the duration of the study.

RESULTS

Patient Characteristics. Table 1 details the characteristics of the 28 patients with metastatic melanoma who initiated treatment with low-dose IL-2 and R24. There were 14 males and 14 females. The median age was 54 years (range, 24–74 years). Eighteen patients had primary cutaneous melanoma, 5 patients had metastases from ocular melanoma, and 5 patients had no primary site identified. The median time from initial diagnosis to treatment was 33 months with a broad range (2–360 months). Sites of metastases included liver (n = 9), lung (n = 9), skin/soft tissues (n = 7) lymph nodes (n = 6), bone (n = 2), gastrointestinal tract (n = 1), and adrenal (n = 1). More than one-half of the patients had more than one metastatic organ site. Ten patients had not received any therapy for metastatic disease prior to IL-2/R24. Twelve patients had previously received chemotherapy (in conjunction with IL-2 in two instances), 2 patients had undergone prior treatment with vaccines, 1 had prior radiation therapy, and 3 had undergone surgical resection alone.

Table 1 Patient characteristics

Sex	
Male	14
Female	14
Age	54 yrs (median) (range, 24–74 yrs)
Primary site	
Cutaneous	18
Ocular	5
Unknown	5
Interval, diagnosis-R24/IL-2	33 mos (median) (range, 2–360 mos)
Sites of metastases	
Liver	9
Lung	9
Skin/soft tissues	8
Lymph nodes	8
Bone	3
Intestine	1
Adrenal	1
No. of metastatic sites	
1	13
2	12
3	3
Prior therapy for metastatic disease	
Chemotherapy	10
Surgery	3
Immunotherapy	2
Chemoimmunotherapy	2
Radiation therapy	1
None	10

Tolerability of IL-2/R24. Of the 28 patients who began treatment, 18 completed all 8 weeks of therapy. Seven patients were withdrawn during the continuous infusion IL-2 phase and three during the combined IL-2/R24 phase. The number of patients treated at each dose level and the number completing treatment are depicted in Table 2. Reasons for withdrawal were catheter infection (5 patients), disease progression (3), a cutaneous hypersensitivity reaction to IL-2 (1), and IL-2-related pulmonary capillary leak syndrome (1). Toxicities were separated into those associated with continuous infusion IL-2, those related to bolus IL-2, and those attributable to R24 monoclonal antibody (Table 3).

During the low-dose continuous infusion IL-2, four patients required temporary interruption of treatment due to fevers >40°C unrelated to detectable infection accompanied by significant malaise. These patients were restarted at a reduced dose of continuous infusion IL-2 (3×10^5 units/m^2/day) and tolerated subsequent therapy. Four patients developed elevated thyroid hormone levels with depression of thyroid-stimulating hormone. Two of these patients became symptomatic and required temporary administration of β-blockers while continuing IL-2. Thyroid function tests returned to normal almost immediately after completion of IL-2 treatment. One patient with ocular melanoma treated at dose level 1 developed hyperkalemia, hyperuricemia, elevated serum creatinine, and elevated lactic dehydrogenase associated with tumor lysis syndrome and tumor shrinkage 2 weeks following completion of all therapy. All

Table 2 IL-2/R24 treatment doses

Dose level	C.I.[a] IL-2[b]	Bolus R24[c]	Bolus IL-2[b]	N	Withdrawn		Completed
					pre-R24	post-R24	
1	4.5	5		8	2	0	6
2	4.5	5	2.5	7	2	1	4
3	4.5	15	2.5	6	2	2	2
4	4.5	5	5.0	7	1	0	6

[a] C.I., continuous infusion.
[b] $\times 10^5$ units/m^2.
[c] mg/m^2.

Table 3 Toxicity of IL-2/R24

	N
Continuous infusion IL-2	
Patients at risk	28
Fatigue	13
Nausea	6
Catheter infection	5
Weight gain	5
Fevers >40°C	4
Hyperthyroidism	4
Ileus	1
Capillary leak syndrome	1 (fatal)
Bolus IL-2 (both dose levels)	
Patients at risk	21
Fevers >40°C	9
Rigors	8
Nausea	7
Decrease systolic BP >20 mmHg	5
Myalgias	5
R24 bolus (both dose levels)	
Patients at risk	21
Urticaria	10
Chest/abdominal pain	9
Fever	8
Rigors	7
Nausea/vomiting	6
Increase systolic BP >20 mmHg	5
Tumor lysis syndrome (elevated potassium, uric acid, creatinine, lactate dehydrogenase 2 weeks posttherapy)	1

Table 4 Continuous infusion IL-2 and peripheral blood lymphocytes

Lymphoid Subset	Pre-Therapy[a]	Week 7/8[b]
NK cells		
CD56+ CD16+	119 ± 21	1098 ± 99 × 10^6 cells/liter
CD56+ CD16−	65 ± 15	323 ± 58
CD56+ IL2Rα+	7 ± 2	49 ± 10
CD56+ IL2Rα−	170 ± 24	1343 ± 122
CD56+ IL2Rβ+	104 ± 30	1206 ± 133
CD56+ IL2Rβ−	78 ± 12	211 ± 31
CD56+ ICAM1+	37 ± 10	434 ± 92
CD56+ ICAM1−	129 ± 17	1038 ± 101
T lymphocytes		
CD3+ CD4+	644 ± 59	898 ± 121
CD3+ CD8+	344 ± 48	396 ± 96
CD3+ IL2Rα+	70 ± 15	248 ± 48
CD3+ IL2Rα−	1008 ± 75	1168 ± 132
CD3+ IL2Rβ+	40 ± 11	80 ± 15
CD3+ IL2Rβ−	1047 ± 90	1381 ± 102
CD3+ ICAM1+	61 ± 10	110 ± 23
CD3+ ICAM1−	1045 ± 129	1188 ± 151

[a] Twenty patients evaluated.
[b] Thirteen patients evaluated.

metabolic abnormalities resolved with i.v. hydration. One patient with widely metastatic disease developed progressive dyspnea during her third week of therapy. She was hospitalized, and IL-2 was discontinued at day +21 of treatment when her chest X-ray demonstrated increased interstitial markings and a unilateral pleural effusion. Despite discontinuation of treatment and aggressive management, the patient developed frank respiratory failure and expired. No postmortem was obtained. It is unclear to what extent IL-2 contributed to her death.

IL-2 administered by 2-h bolus at the low doses of 2.5 or 5×10^5 units/m^2 was generally well tolerated although symptoms were common in these patients and included fever (>40°C), nausea, chills, myalgias, and fatigue (Table 3). Hypotension requiring more than 2 liters of fluid for resuscitation was uncommon. No patients required hospital admission.

R24 was generally well tolerated at the initial dose of 5 mg/m^2. Of the 18 patients who received R24 at this dose, 7 experienced temporary chest tightness and abdominal cramps. ECGs were always unchanged from baseline, and discomfort resolved within 1–2 h. Urticaria was also noted in eight patients, although it did not occur after each dose of R24 in these individuals. Fevers and chills were observed in five patients, nausea and vomiting were noted in four patients. Three patients developed a >20-mm increase in systolic blood pressure, which returned to normal within 90 min without intervention. The higher dose of R24 (15 mg/m^2) produced severe abdominal/chest pain in two of four patients. Pain was deemed sufficiently severe that these patients were not rechallenged at these doses, and subsequent patients were treated at the lower R24 dose of 5 μg/m^2. Thus, in patients receiving 4.5×10^5 units/m^2/day of continuous infusion IL-2, R24 dose was not escalated above 5 mg/m^2.

Immunological Effects. Low-dose continuous infusion IL-2 progressively expanded the number of circulating CD16+ NK cells during the 8 weeks of therapy from an initial average value of 119 ± 21 to 1098 ± 99 × 10^6 cells/l ($P = 0.001$) at the completion of treatment (Table 4). The majority of these NK cells expressed the p75 β chain of the IL-2R but not the p55 α chain (CD25). The number of NK cells expressing adhesion molecules such as ICAM-1 (CD54) increased as well, rising from 37 ± 10 × 10^6 cells/liter initially to 434 ± 92 × 10^6 cells/liter ($P = 0.04$). A less pronounced (approximately 25%)

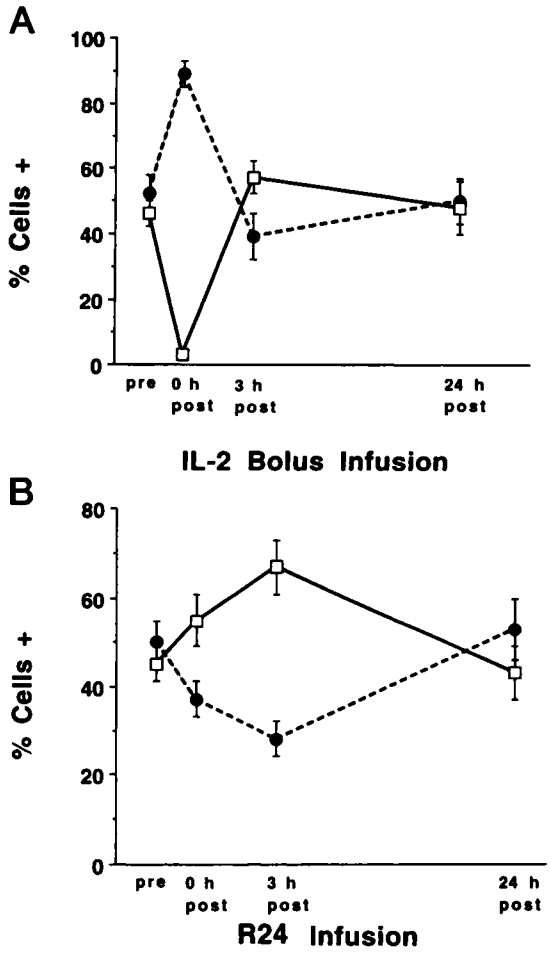

Fig. 2 Immunological effects of IL-2 and R24 boluses on NK cells and T cells. The effect of IL-2 (*A*) or R24 (*B*) boluses on the fraction of circulating CD56+ NK cells (□) and CD3+ T cells (●) in patients receiving continuous infusion low-dose IL-2 is depicted. Levels immediately before and after bolus administration as well as 3 and 24 h after treatment are shown. Values represent a mean (*bars*, SE) of 14 patients tested at the time of their first set of boluses in week 5.

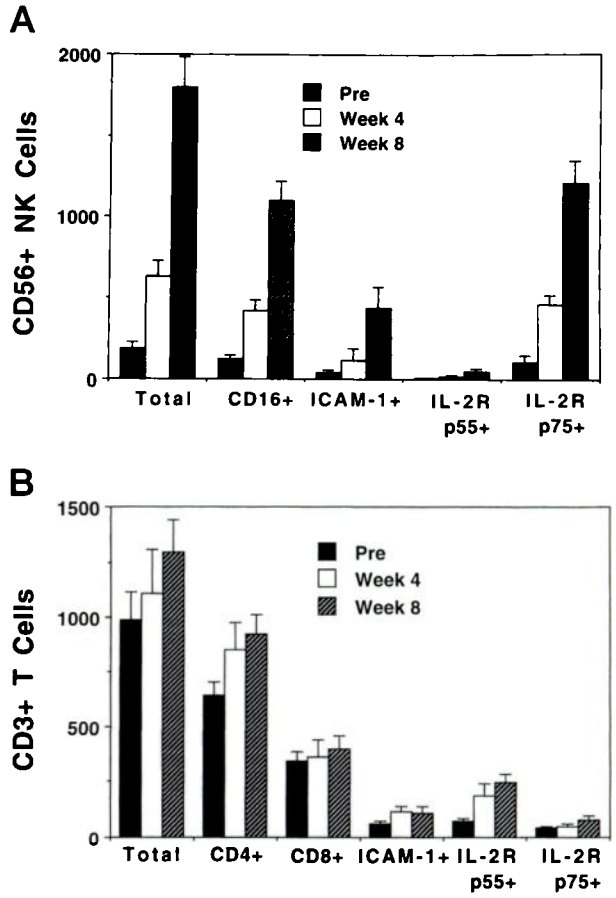

Fig. 3 Effect of continuous infusion IL-2 treatment on NK cell and T-cell subsets. The total mean number (*bars*, SE) of NK cells (*A*) and T cells (*B*) is displayed in patients prior to therapy (week 0; 20 patients), during continuous infusion IL-2 before bolus therapy is initiated (week 4; 14 patients), and at the end of continuous infusion therapy after all R24 and IL-2 boluses have been administered (week 8; 13 patients). Surface expression of several antigens on NK cells and T cells is also displayed at these time periods. Significant increases in total NK cells ($P = 0.008$, 0–4 weeks; $P = 0.04$, 4–8 weeks), CD16+ NK cells ($P = 0.04$, 0–4 weeks; $P = 0.05$, 4–8 weeks), and NK cells expressing IL-2R β chain ($P = 0.01$, 0–4 weeks; $P = 0.02$, 4–8 weeks) were observed without significant changes in T-cell subsets during these periods.

increase in T lymphocytes was observed after 8 weeks of therapy ($P = 0.28$). There was a modest increase in the number of circulating T cells expressing the low affinity α chain of the IL-2R ($70 \pm 15 \times 10^6$ cells/liter to $248 \pm 38 \times 10^6$ cells/liter; $P = 0.18$). Treatment did not influence the number of circulating B lymphocytes in these patients.

Boluses of R24 and IL-2 appeared to have significantly different effects upon lymphoid subsets in these patients. IL-2 boluses, even at doses as low as the ones used in this study, produce an immediate and dramatic drop in the number of lymphocytes that recovered within 24 h. When the peripheral blood compartment was examined directly after IL-2 bolus infusion, there was almost a complete absence of NK cells (Fig. 2*A*). It is presumed that the IL-2 bolus can saturate the intermediate affinity receptors on the NK cell surface, activate those cells, up-regulate adhesion molecules, and induce temporary margination (22–24). Resting T cells, which generally lack such IL-2 receptor expression, are far less affected. In contrast, bolus R24, which reacts with G_{D3} moiety on the surface of a subset of T lymphocytes, but not NK cells, resulted in a relative decline in the fraction of circulating T cells that returned to pre-R24 bolus values at 24 h (Fig. 2*B*). The introduction of R24 and IL-2 boluses in the fifth and sixth weeks did not appear to interfere with progressive NK cell expansion observed. The relative increase in CD16+ NK cells during the combined bolus/continuous infusion phase (weeks 4–8) was similar to that during the continuous infusion phase (weeks 0–4) alone (Fig. 3).

Cytolytic Activity. We demonstrated previously that low-dose IL-2 administration increases cytolytic activity against NK-sensitive (K562) tumor targets in 4-h chromium release assays. In six patients evaluated on this study at the beginning of the fifth week of therapy (prior to the first R24 bolus), cytolytic activity against K562 was increased from $14 \pm 4\%$ to $65 \pm 10\%$

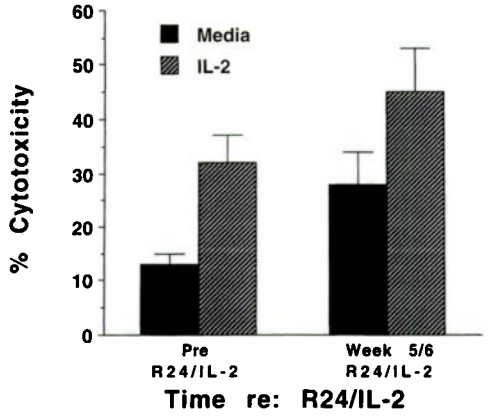

Fig. 4 ADCC of melanoma tumor targets. Lysis of SK-MEL-28 cells in the presence of R24 by PBMCs obtained from six patients in the fifth or sixth week of therapy is shown and compared to lysis induced by PBMCs from these patients prior to treatment. Cytotoxicity was assessed in 4-h chromium release assays at an E:T ratio of 20:1. Assays were performed after an 18-h incubation in media alone or media supplemented with IL-2 (500 units/ml). Values represent the mean (*bars*, SE) percentage target lysis of the six patients.

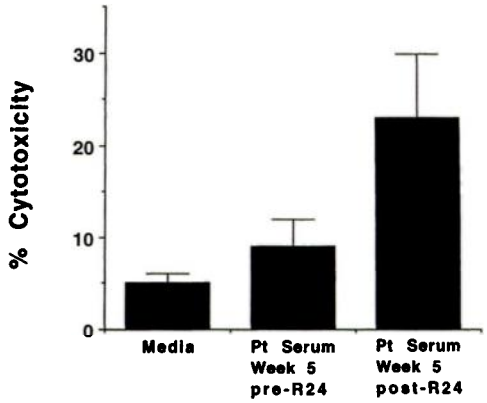

Fig. 5 Effect of serum from patients on cytolysis of melanoma tumor targets. Killing of SK-MEL-28 cells by PBMCs from five patients obtained during week 5 of continuous infusion IL-2 after R24 bolus is displayed. Four-h chromium release assays were performed at an E:T ratio of 20:1 in the presence of media, serum was obtained from corresponding patients before R24 bolus, and serum was obtained from patients following R24 bolus. *Bars*, SE.

at a 20:1 E:T ratio. The ability to destroy SK-MEL-28 melanoma tumor targets in the presence of exogenous R24 was also increased after 5–6 weeks of therapy ($P = 0.08$). When cellular effectors obtained from patients were preincubated with exogenous IL-2 to mimic *in vitro* the *in vivo* bolus schema, cytolytic activity increased further still (Fig. 4).

Cytotoxicity against SK-MEL-28 targets was also assessed before and after R24 bolus in three patients. Cytolytic activity of patient PBMCs obtained immediately following R24 bolus was not significantly different than that of PBMCs obtained immediately prior to R24 bolus (data not shown). Although there did not appear to be a direct effect of R24 bolus on cytolytic activity of cellular effectors, R24 treatment did induce a biological change detectable in patient serum. A significant increase in killing of melanoma targets was observed when assays were performed in the presence of serum obtained from patients following R24 bolus compared to assays performed with either media alone or with patient serum obtained immediately prior to R24 bolus (Fig. 5), suggesting that R24 bolus infusions produced antibody concentrations sufficient to augment destruction of melanoma targets at least *in vitro*.

Tumor Response. Of the 18 patients who completed all 8 weeks of therapy, there were three patients who experienced responses. One of these was a partial response. This patient with ocular melanoma and extensive (>20) liver metastases experienced tumor lysis 1 week after completing therapy characterized by hyperkalemia, hyperuricemia, elevated lactic dehydrogenase, and elevated serum creatinine. He responded to aggressive hydration. Follow-up computed tomography scan demonstrated a ≥80% reduction in the size of his numerous liver metastases. He remained progression free for 5 months but then had evidence of tumor growth. Two other patients (one again with ocular melanoma) had evidence of a 25–50% reduction in tumor size (lung and liver). Both were retreated with IL-2/R24 but had no further response. All three had received R24 at 5 mg/m^2. One received no bolus IL-2 (dose level 1), one received IL-2 bolus at 2.5×10^5 units/m^2 (dose level 2), and one received IL-2 bolus at 5.0×10^5 units/m^2 (dose level 4). The relatively small number of responders did not permit correlation with levels of NK or T-cell subsets generated during the study. Of the remaining 15 patients who completed therapy, 8 had stable disease and 7 had evidence of significant progression following completion of treatment. The brain was the primary site of treatment failure in four patients. Two of the eight patients with stable disease experienced a near complete response when subsequently treated with dacarbazine at standard doses.

DISCUSSION

The capacity to preferentially target tumor cells by monoclonal antibodies has created and sustained interest in using these agents in the treatment of patients with metastatic cancer (2–5). The mechanisms by which unconjugated antibodies exert their antitumor effects clinically are not clear. Unconjugated antibodies may act by cell surface binding followed by opsonization, complement fixation, and lysis, or alternatively, by facilitating destruction mediated by cytotoxic effector cells (15–17, 25–27). Strategies designed to augment effector cell function and promote destruction of antibody-coated tumor cells might produce improved antitumor responses.

The schedule implemented in this study allows selective expansion of a specific population of effectors (NK cells) that have the capacity to destroy antibody-coated tumor targets. Once these NK effectors are expanded, they can be activated with a bolus dose of additional IL-2 following infusion of the targeting R24 antibody. NK effector cells, once primed by low-dose continuous infusion IL-2 *in vivo*, become exquisitely sensitive to further stimulation by IL-2 (19–21). Thus, activation of these NK effectors can be safely achieved using a low-bolus dose of IL-2. IL-2 bolus stimulation results in upregulation of ICAM-1 and other adhesion molecules on NK cells which then, presumably, leads to cellular margination and

perhaps extravasation (22, 28). The temporary disappearance of CD56+ effector cells from the blood of patients following IL-2 boluses confirms that the low doses of IL-2 we used are capable of activating these NK cells without the toxicity usually associated with higher doses of IL-2 (29, 30).

Although the clinical responses we observed in this study were only modest, the activity in patients with ocular melanoma is intriguing. Uveal melanomas do react with R24 antibody (31). Two of five patients with ocular melanoma and hepatic metastases had demonstrable responses. Given the notorious refractoriness of ocular melanoma to chemotherapy and other therapeutic initiatives, exploration of the potential role of R24/IL-2 in the treatment of these patients merits further consideration. Future studies evaluating this treatment approach should be undertaken, concentrating on ways to promote the interaction between activated effector cell populations and antibody-labeled targets. One approach might involve altering the timing of R24 administration. Since the number of NK cells progressively increased in our patients throughout the 8-week course of continuous infusion IL-2, it might be reasonable to give R24 antibody infusions later in the treatment course when the number of these cytotoxic effectors is greater. Because low-dose IL-2 is well tolerated, treatment with IL-2 to expand NK effectors could be extended even beyond the 8-week course to further increase NK effector cell number. In prior clinical studies of IL-2 and R24, IL-2 was administered at considerably higher doses for only a limited duration (32, 33). In future studies, administering IL-2 s.c. rather than i.v. during the "priming" phase may reduce the likelihood of treatment interruption and allow more prolonged treatment by eliminating the likelihood of catheter infections. Providing more intensive exposure to R24 during IL-2 administration may also prove worthwhile. However, because R24 is a murine antibody, repetitive treatments may be limited by induction of human antimouse antibodies. Therefore, studies of humanized R24 antibody may prove valuable (34, 35).

Other effector populations in addition to NK cells may merit investigation as well. NK cells are not the only leukocytes capable of mediating ADCC. Monocytes, granulocytes, and other effectors may have the capacity to kill antibody-labeled cells. Efforts to expand and activate these cells with other cytokines have been undertaken in patients receiving R24 therapy for metastatic melanoma. Granulocyte/macrophage-colony-stimulating factor, tumor necrosis factor, and IFN-α have been combined with R24 in clinical studies (36–39). Although effector cell expansion has been demonstrated with these cytokines, clinical responses have thus far been rare in these preliminary studies.

Whereas it has been presumed that the clinical responses observed in patients with melanoma after R24 infusion are related to the binding of the antibody to tumor cells, it is also possible that other mechanisms may be operating. R24 not only recognizes G_{D3} on melanoma cells, but it also reacts with the G_{D3} moiety on 15–25% of peripheral T lymphocytes. It has been demonstrated that ligation of G_{D3} by R24 can cause proliferation of T cells and enhance cellular cytotoxicity (10, 40, 41). Incubation with R24 can also induce secretion of many cytokines including IL-1, IL-2, and TNF (40, 41). Moreover, G_{D3} ligation by R24 provides a costimulatory signal that can promote antigen-dependent T-cell clonal responses in the absence of costimulation provided by the B7 molecule (42, 43). Furthermore, primary stimulation of T cells by R24 can prevent induction of clonal anergy under conditions when the B7:CD28 interaction is blocked by CTLA4-immunoglobulin (42). The mechanism by which R24 exerts these effects is not completely understood, but it appears to be IL-2 dependent, because coincubation with anti-IL2 neutralizing antibodies or antibodies to IL-2R will block these actions on T cells (42). The effect of R24 on T cells was suggested in our study by the sudden decrease in the percentage of T cells in the peripheral blood of patients immediately following R24 infusion. Further exploration of the effects of G_{D3} ligation by R24 on T cells may provide new clues as to how this antibody might be of more clinical utility in antitumor therapy and may, in fact, provide another distinct rationale for its combination with IL-2 in future studies.

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