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Interleukin-2

James W. Mier, MD
and Michael B. Atkins, MD

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1. INTRODUCTION

Interleukin-2 (IL-2) is the first agent available for the treatment of metastatic cancer that functions solely through the activation of the immune system. IL-2 enhances several aspects of cellular immune function and induces tumor regression in numerous murine tumor models. Clinical investigations with recombinant IL-2 have demonstrated that it possesses significant antitumor activity, producing durable complete responses in a small percentage of patients with either metastatic melanoma or renal cell carcinoma. The high-dose intravenous bolus IL-2 regimen developed at the National Cancer Institute (NCI) Surgery Branch received Food and Drug Administration (FDA) approval for the treatment of metastatic renal cell carcinoma in 1992 and malignant melanoma in 1998. This chapter will review the *in vitro* effects of IL-2, the results of pertinent preclinical studies, and subsequent clinical investigations of IL-2-based regimens in patients with malignant melanoma.

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1.1. IL-2 and Its Receptor

In 1976, the conditioned medium of lectin-stimulated human peripheral blood mononuclear cells (PBMCs) was used to propagate indefinitely pre-activated human T cells *ex vivo* (1). This initial description of what was then termed T cell growth factor (TCGF) was followed by the isolation, biochemical characterization, and ultimately, the cDNA cloning of the responsible lymphokine (2,3). Subsequently designated IL-2, this factor was shown to be a 15-kDa polypeptide made up of 153 amino acids, the first 20 of which are proteolytically cleaved during secretion. Crystallographic analysis indicates that IL-2 is a spherical molecule comprised of 6 α helical regions.

The various effects of IL-2 are mediated through its binding to specific surface receptors (reviewed in ref. 4). As is the case with IL-2 itself, the expression of high affinity IL-2 receptors (IL-2Rs) is induced in T cells as a result of an encounter with an antigenic peptide/major histocompatibility complex (MHC), which accommodates the clonotypic T cell antigen receptor (TCR). With the exception of a minor population of memory T cells, which presumably were activated *in vivo* by a prior antigen exposure, freshly isolated peripheral blood T cells do not constitutively express high affinity IL-2Rs (5).

The high affinity IL-2R consists of three distinct subunits designated the α , β , and γ chains (Fig. 1). The α chain (6) is a 251 amino acid polypeptide with a large extracellular domain capable of binding IL-2 with low affinity, a transmembrane span, and a short 13 residue cytoplasmic tail, which is not involved in IL-2-induced signaling. The β chain has a 214 amino acid extracellular domain, a transmembrane motif, and a large 286 residue cytoplasmic tail absolutely essential for IL-2 signaling (7). The IL-2R- β chain has paired cysteines at two sites within the extracellular domain and a perimembrane WSXWS motif, both of which are signature motifs of an enlarging cytokine receptor superfamily, which includes the receptors for IL-3, IL-4, IL-6, IL-7, granulocyte-macrophage colony-stimulating factor (GM-CSF), prolactin, erythropoietin, and growth hormone.

The novel 64 kDa γ chain is another member of the cytokine receptor superfamily (8), which, when paired with the β chain, binds IL-2 with intermediate affinity. This receptor chain is shared by the receptors for several lymphokines, including IL-4, IL-7, IL-9, and IL-15, as well as IL-2 (9). Mutations in the gene encoding this receptor chain account for most, if not all, cases of X-linked severe combined immunodeficiency (10). The three chains together yield a high affinity IL-2R, which transduces signals and is internalized in response to IL-2 binding. Resting T cells constitutively express low levels of the IL-2R- γ chain, but not the α or β chains. All three chains are up-regulated as a result of antigenic stimulation. Resting natural killer (NK) cells constitutively express the β chain, and both the α and γ chains are induced in these cells by exposure to IL-2 or IL-12 (11).

Fig. 1. The high affinity IL-2R and associated signaling pathways. The high affinity IL-2R is a heterotrimer consisting of α , β , and γ chains, the latter two of which are critically involved in the transmission of signals activated by IL-2 binding. Signaling pathways activated include several STATs and src-related protein tyrosine kinases, which are responsible for cell cycle progression, the regulation of motility, and susceptibility to apoptosis.

IL-2 induces the tyrosine phosphorylation of numerous cellular proteins, including the IL-2R- β chain itself. These events are transduced through kinases such as the *src* family member $p56^{lck}$ that physically associate with the cytoplasmic domains of the receptor subunits (Fig. 1) (12,13). IL-2 also induces the recruitment and subsequent tyrosine phosphorylation of the adapter protein *Shc* to the IL-2R- β chain. This particular association is thought to be largely responsible for the activation of $p21^{ras}$ and the downstream microtubule-associated protein (MAP) kinases *erk-1* and *erk-2* in response to IL-2 (14). In addition to the association with *src* family tyrosine kinases, both the β and γ receptor chains associate with members of the Janus kinase (JAK) family of tyrosine kinases (15). JAK3, for example, associates with the IL-2R- γ chain, whereas both JAK1 and JAK2 associate with the β chain. JAKs activate various members of the signal transduction and activators of transcription (STAT) family of transcription factors. The binding of IL-2 to its receptor on antigen-primed T cells results in the phosphorylation and activation of STAT1, STAT3, and STAT5. In NK cells, STAT4 is also activated by IL-2 (16).

1.2. In Vitro Effects of IL-2

In addition to its proliferative effects, IL-2 induces the synthesis of an array of secondary cytokines such as IL-1, tumor necrosis factor (TNF), IL-6, and lymphotoxin (17), several of which are detectable in the circulation of cancer patients receiving IL-2 and thought to contribute to IL-2-induced tumor regression and toxicity (18). The biological effect of IL-2, arguably most pertinent to its use as an antitumor agent, may be its ability to enhance the cytolytic activity of antigen-specific cytotoxic T lymphocyte (CTL) cells and NK cells (19,20). In addition to increasing the cytolytic activity of these cells for their respective targets, IL-2 markedly diversifies the range of target cells these effectors are able to kill (21,22). Indeed, human peripheral blood lymphocytes exposed only to high concentrations of IL-2 without prior exposure to tumor cells are able to kill virtually all tumor cell lines and most freshly isolated tumor cells in vitro regardless of the particular human leukocyte antigen (HLA) class I alleles expressed by the target cell. The cells responsible for this HLA-unrestricted killing in response to IL-2 have been termed “lymphokine-activated killer” or LAK cells (21,22) and appear to be a mixture of activated NK cells and CD3⁺/CD8⁺ cytotoxic T cells. As described below, these ex vivo-activated LAK cells featured prominently in the early clinical trials carried out with IL-2 in cancer patients.

1.3. Preclinical Studies with IL-2 in Tumor-Bearing Mice

IL-2 has been extensively evaluated as an antitumor agent in a variety of murine tumor models. The lymphokine has been used alone, in combination with other cytokines, and in conjunction with various adoptively transferred, ex vivo-activated lymphoid preparations to eradicate a wide range of local and metastatic tumors. Early studies demonstrated that IL-2 used alone could reduce or eliminate pulmonary metastases from melanoma and methylcholanthrene-induced sarcoma cell lines and that this antitumor effect was strictly dependent on the dose of IL-2 administered (23). In more recent studies, in which mice were immunized with dendritic cells pulsed with tumor lysates, the concurrent systemic administration of IL-2 was shown to enhance the efficacy of the vaccine (24). In several studies, the effects of IL-2 could be enhanced by the concurrent administration of LAK cells generated by culturing splenocytes ex vivo in IL-2-containing media (25). Mice bearing B16 melanoma pulmonary metastases, for example, are highly responsive to treatment with the combination of IL-2 and LAK cells (26). Lymphocytes present within tumor infiltrates are enriched for effector cells capable of killing tumor cells (27,28), and, when isolated and tested in vitro for cytolytic activity against autologous tumor cells, these tumor-infiltrating lymphocytes (TILs) are 50- to 100-fold more potent than IL-2-activated splenocytes (LAK cells). Several studies have demonstrated the efficacy of TIL/IL-2 in the eradication of disseminated malignancy (27,28).

2. CLINICAL APPLICATIONS OF IL-2

2.1. High-Dose IL-2 Administered as Intravenous Bolus Injections

The encouraging results of studies with tumor-bearing mice prompted the rapid movement of IL-2 into the clinical setting. Early clinical trials were initiated prior to the availability of recombinant material and involved modest doses of highly purified T cell-derived IL-2, the administration of which produced transient fever and lymphopenia, but no sustained ill effects or tumor responses (29,30). The advent of recombinant IL-2 allowed much higher doses to be administered. In order to facilitate comparisons between the various studies reviewed in this and subsequent sections, we have listed all IL-2 doses in International Units (IU) using the following conversion ratios: 1 Cetus Unit = 6 IU; 1 Hoffman-LaRoche Unit = 1 Biologics Response Modifier Program (BRMP) Unit = 2.6 IU (31).

The high dose intravenous bolus IL-2 therapy currently in use at many institutions was initially pioneered by the NCI Surgery Branch in the mid-1980s. This regimen was based on data from previously cited murine studies demonstrating that tumor responses to IL-2 were dose-dependent and enhanced when the daily dosage of IL-2 was divided over 3 daily doses or when IL-2 was combined with LAK cells. The early clinical trials performed at the NCI Surgery Branch established the toxicity, management guidelines, and maximum tolerated dose for the high-dose bolus regimen when administered either alone or in combination with autologous LAK cells (32,33). In the regimen developed at the NCI, IL-2 (Cetus/Chiron) was administered at 600,000–720,000 IU/kg intravenously every 8 h for d 1–5 and 11–15 of a treatment course. In those cases in which LAK cells were given, the cells were prepared from leukapheresis-derived PBMCs obtained on d 8,9, and 11 and administered with IL-2 on d 12,13, and 15. A maximum of 28–30 IL-2 doses per course was administered; however, doses were frequently withheld for excessive toxicity. Treatment courses were repeated at 8- to 12-wk intervals in responding patients. This IL-2 regimen administered either alone or in combination with LAK cells produced overall tumor responses in 15 to 20% of patients with metastatic melanoma in clinical trials conducted at either the NCI Surgery Branch or within the Cytokine Working Group (formerly Extramural IL-2 and LAK Working Group). Complete responses were noted in 4–6% of patients and were frequently durable (30,34–37).

Long-term follow-up data are now available on metastatic melanoma patients that participated in these early high-dose bolus IL-2 trials. These data confirm the efficacy of IL-2 in at least a minority of patients. We recently analyzed data on 270 patients that were treated on the 8 high-dose IL-2 trials conducted between 1985 and 1993 (37,38). Data were initially analyzed through the fall of 1996 (37) and then updated through December, 1998 (38). Although this study population was highly selected for excellent organ function and the absence of central nervous system (CNS) metastases or significant cardiovascular disease, it included

Table 1
High-Dose Intravenous Bolus IL-2 Alone Therapy

<i>Author (reference)</i>	<i>Dose</i>	<i>Patients no: evaluable</i>	<i>Response</i>		<i>Total (%)</i>	<i>Comments</i>
			<i>CR</i>	<i>PR</i>		
Atkins et al. (67)	600,000–720,000 IU/kg IL-2 (Chiron) IV every 8 h d 1–5 and 15–19.	305	19 (6%)	28 (9%)	47/305 (15%)	5 patients received 360–540,000 IU/kg of IL-2.

IL-2, Interleukin-2; IV, intravenous; IU, International Units.

Adapted from Atkins, Shet, and Sosman (67).

a large percentage of individuals with other poor prognostic features such as visceral metastases, multiple metastatic sites, and/or prior systemic therapy. Objective responses were seen in 43 (16%) patients (95% confidence interval [CI]: 12 to 21%) including 17 (6%) complete responses and 26 (10%) partial responses (Table 1). Responses were noted in all disease sites, including lung, liver, lymph nodes, soft tissue, adrenal, bone, and subcutaneous tissue, and were observed in some patients with large tumor burdens or bulky individual lesions. Ten of 17 complete responses (59%) and two of 26 partial responses (8%) were ongoing at >42 to >122 mo. Progression-free survival for these 12 patients ranged from >70 to >150 mo. The median duration of response for all responders was 8.9 mo (range 1.5 – >122 mo), and 28% of these responding patients remained free of disease progression (Fig. 2). The median duration of complete responses had yet to be reached and exceeded 59 mo. No patient responding for longer than 30 mo exhibited disease recurrence or progression, suggesting that some patients may actually have been “cured” of their disease.

Responses were less frequent in patients with baseline Eastern Cooperative Oncology Group (ECOG) performance status of 1 or greater or in those who had received prior systemic therapy. Involvement of multiple organ sites or visceral metastases did not reduce the likelihood of treatment benefit. Five additional responding patients (1 complete response [CR] and 4 partial response [PR]), who developed an isolated site of progression that was amenable to local therapy, were alive and free of disease at >79 and >104 mo following IL-2 therapy, suggesting a role for regional salvage treatment in patients who develop isolated site relapse after a response to IL-2 therapy. Overall, 31 (11%) were alive at last contact. The median survival (Kaplan-Meier) for the entire treated population was 12 mo (Fig. 3). Nineteen (44%) of the responding patients remained alive, all having survived more than 5 yr. The six deaths (2.2%) that occurred during these trials were all related to infections and occurred prior to 1991 (39), after which prophylactic antibiotics were routinely incorporated into the treatment protocol.

Fig. 2. Kaplan-Meier estimate of response durations for patients with metastatic melanoma exhibiting a complete or partial response to high-dose intravenous bolus IL-2. Adapted with permission from ref. 38.

Fig. 3. Kaplan-Meier estimate of overall survival for patients with metastatic melanoma treated with high-dose intravenous bolus IL-2 (N = 270). Adapted with permission from ref. 37.

Rosenberg et al. has reported long-term follow-up data on 409 patients (182 with metastatic melanoma, 217 with renal cell carcinoma) treated with high-dose intravenous bolus IL-2 alone at the NCI Surgery Branch (40) between Septem-

ber, 1985 and November, 1996. All patients received 720,000 IU of IL-2 (Cetus/Chiron) every 8 h for a maximum of 15 doses per cycle as clinically tolerated. Some of these patients received a polyethylene glycol (PEG)-conjugated form of IL-2 during the second treatment cycle. These patients fared no differently than those receiving conventional high-dose bolus IL-2 (41). All but 35 of these patients were included in the data set described above (*see* Table 1). Complete regression was observed in 12 (6.6%) of the 182 patients with metastatic melanoma; partial regression occurred in 15 (8.2%) patients. Ten (6%) patients with melanoma exhibited ongoing continuous CR from 83 to 161 + mo from the onset of treatment. In this population, CR was significantly more frequent in patients who had not received prior immunotherapy. Only 2% of previously treated patients achieved a CR. The CRs tended to occur in patients that received a relatively large fraction of the scheduled doses and whose lymphocyte counts had the highest rebound at the completion of the first cycle of IL-2.

2.2. IL-2 Administered at Lower Doses or by Alternative Schedules

The toxicity of high-dose intravenous bolus IL-2 and the expense associated with the obligatory hospitalization of patients receiving this form of therapy have provided impetus for the development of alternative regimens using lower doses of IL-2. Several such regimens involving IL-2 administered either by lower dose intravenous bolus, continuous infusion, or subcutaneous injection have since been evaluated. Generally, treatment schedules using lower doses of IL-2 allow more prolonged treatment. IL-2 administered this way expands the number and enhances the function of circulating NK cells (42,43). Antibody-dependent cellular cytotoxicity (ADCC) and NK/LAK cell activity are increased by relatively low and less toxic doses of IL-2 (42–45). Unfortunately, these immunologic parameters do not correlate with the antitumor effect observed, which in the case of melanoma are often inferior to those achieved with high doses. The results of these various high dose and intermediate dose continuous infusion IL-2 alone regimens, in patients with metastatic melanoma, are summarized in Table 2 and described in detail below.

The alternative IL-2 regimen is currently the most widely used. This treatment consisted of IL-2 administered at 18 MIU/m²/d by continuous infusion on d 1–5 and 11–15 with or without LAK cells (46). The IL-2/LAK cell regimen produced tumor responses in 5 of 10 (50%) of evaluable patients with melanoma (46). Toxicity was still significant, but generally felt to be more predictable and manageable than with the high-dose intravenous bolus IL-2 schedule developed by the NCI Surgery Branch. The National Biotherapy Study Group (NBSG) studied this continuous infusion IL-2 regimen omitting the LAK cells. Objective responses were seen in 33 of 188 (18%) patients with melanoma (47). An alternative schedule, which combined bolus and continuous infusion IL-2, was subsequently explored. In this schedule, a total of 90 MIU/m² of IL-2 was administered over

Table 2
Continuous Infusion and Low-Dose IL-2 Alone Regimens

<i>IL-2 Administration route/dose level</i>	<i>N</i>	<i>CR plus PR</i>	<i>Response rate (%)</i>
High-dose continuous infusion	219	40	18
Hybrid (bolus plus continuous infusion)	18	4	22
Low-dose IV or subcutaneous	95	1	1

Adapted from Atkins, Shet, and Sosman (67).

3 instead of 5 d (48). Treatment was administered every 2 wk for 2 mo. Twenty-two patients with melanoma were enrolled, of which 18 had measurable disease. Two patients achieved a CR and another two were PRs (22% overall response rate [RR]; 95% CI of 6–48%). Additional studies of this regimen and long-term follow-up data on the first patients to receive this form of immunotherapy have yet to be reported.

A slightly lower dose of IL-2 (12 MIU/m²/d) administered by continuous infusion for 4 d on 4 consecutive wk (49). Out of 31 patients, 1 CR and 6 PR for a 22% overall RR (10–41%; 95% CI) resulted. Toxicity was sufficiently mild to allow these patients to be managed on a general hospital ward. Responses lasted from 4–18+ mo. Finally, a regimen using IL-2 at 20 MIU/m² for d 1–5, 15–18, and 29–31 was investigated. While toxicity was significant, 8 responses were reported among 24 patients with metastatic melanoma (50). However, 4 of the responses were very transient, lasting less than 1 mo. In summary, the available data suggest that continuous infusion regimens may be comparable to high-dose bolus IL-2 in terms of response rates. However, the long-term follow-up experience with these regimens raises concerns about the durability of these responses. In one study, only 10 of 175 patients (6%) with melanoma and only 25% of the complete responders survived for greater than 3 yr (51).

Several investigators have evaluated still lower dose regimens, which could be safely administered in an outpatient setting. The results achieved with various low dose IL-2 treatment regimens are summarized in Table 2. For example, a low-dose outpatient regimen in which IL-2 was administered at 1.8 MIU/m²/d by continuous infusion, 24 h/d for 3 consecutive wk (21 d) (52). While toxicity was quite manageable, no responses were observed in the 15 melanoma patients included in their phase II study (53). The Southwest Oncology Group (SWOG) recently explored a regimen in which low doses of IL-2 were administered weekly by bolus injection to patients with metastatic melanoma (54). This regimen produced significant short-term side effects but virtually no antitumor activity. Together, these results suggest that the minimum dose of IL-2 capable of inducing disease regression in even a minority of melanoma patients may exceed that which could be safely administered in an outpatient setting. Very low dose,

outpatient IL-2 regimens appear to be completely inactive in metastatic melanoma. Although conventional high-dose bolus IL-2 has not been formally compared with less intensive regimens in randomized trials, the frequency, durability, and quality of the responses noted in melanoma patients treated as outpatients are sufficiently discouraging so as to eliminate any incentive to carry out such large-scale phase III trials.

2.3. Toxicity of IL-2 Therapy

The utility of high-dose IL-2 has been limited by its toxicity, many features of which resemble bacterial sepsis. IL-2 side effects are dose-dependent and, fortunately, predictable and reversible. Common side effects include fever, chills, lethargy, diarrhea, nausea, anemia, thrombocytopenia, eosinophilia, diffuse erythroderma, hepatic dysfunction, and confusion (55,56). Myocarditis also occurs in approximately 5% of patients. IL-2 therapy routinely causes a “capillary leak syndrome” characterized by peripheral edema, hypotension, adult respiratory distress syndrome (ARDS), and pre-renal azotemia. As a consequence of these side effects, few patients are able to receive all of the therapy scheduled in high dose regimens. IL-2 produces a neutrophil chemotactic defect that predisposes patients to infection with gram positive and occasionally gram negative bacteria (39,55,56). Prior to the discovery of this defect, high-dose IL-2 treatment was associated with 2–4% mortality, largely related to infection or cardiac toxicity (37,42,55,56). The routine use of antibiotic prophylaxis, more extensive cardiac screening, and the more judicious IL-2 administration have greatly enhanced the safety of this therapy. Since 1990, the mortality rates at experienced treatment centers have been less than 1% (57). Nonetheless, the considerable toxicity of the high-dose IL-2 regimen has continued to limit its application to highly selected patients with excellent performance status and adequate organ function treated at medical centers with considerable experience with this approach.

As expected, lower dose IL-2 regimens have been generally better tolerated. Side effects have consisted of flu-like symptoms, erythroderma, and fluid retention. The more worrisome pulmonary, renal, cardiac, and hepatic side effects are usually not seen in patients receiving low-dose regimens. Unfortunately, this reduction in toxicity may be associated with an even more dramatic fall off in clinical efficacy, limiting the use of such lower dose regimens.

2.4. IL-2 Toxicity Reduction Strategies

IL-2 is a potent inducer of pro-inflammatory cytokines such as IL-1, TNF α , and interferon gamma (IFN γ) (17,18,32,58). These substances and others, including nitric oxide (NO) (59), may play a role in IL-2 toxicity. To the extent that this is the case, one would expect that suppression of the synthesis of these mediators or selective neutralization of their biological activities might enhance the therapeutic index of IL-2 by dissociating its toxic effects from its antitumor effects.

A number of such toxicity dissociation approaches have been tried with limited success. Most of these approaches have utilized the high-dose intravenous bolus IL-2 schedule described previously. The addition of dexamethasone to this regimen, for example, prevented the induction of circulating TNF and significantly reduced treatment-related fever and hypotension, allowing for a threefold increase in the maximum tolerated dose (60). However, potential interference with the antitumor efficacy of IL-2 by glucocorticoids limited this approach (60,61) and highlighted the need for more selective ways of inhibiting the effects of secondary cytokines released in response to IL-2.

More recent investigations have involved the concomitant administration of IL-2 with soluble neutralizing receptors to TNF (sTNFR:IgG) or IL-1 (sIL-1R) or TNF or IL-1 signal transduction inhibitors of the methylxanthine class (e.g., pentoxifylline or CT1501R) (62–65). Several of these approaches showed significant promise in animal models and were able to inhibit some of the biologic effects associated with high-dose IL-2 in patients without interfering with antitumor effects. In fact, responses were seen in 9 (5 CR and 4 PR) of 45 patients (20%) with metastatic melanoma treated as part of these clinical trials (66,67). Unfortunately, none of these agents was able to significantly block IL-2 toxicity as measured by the incidence of hypotension, the use of pressors, or the number of IL-2 doses that could be administered. These results indicate that multiple factors, including a variety of pro-inflammatory cytokines or possibly direct interactions of activated lymphocytes with the vascular endothelium may be responsible for the dose-limiting side effects of IL-2. If such were the case, the neutralization of individual cytokines such as TNF or IL-1 with soluble cytokine receptors would be unlikely to abrogate toxicity.

The tetravalent guanyldiazide compound CNI-1493 blocks the synthesis of numerous pro-inflammatory cytokines such as TNF and IL-1 through the inhibition of the P38 MAP kinase signaling pathway. At high concentrations, it inhibits lipopolysaccharide- and IFN γ -induced NO production by blocking L-arginine uptake (68,69). This compound is selectively taken up by monocytes and, therefore, has no effect on lymphocyte function or activation. Preclinical experiments suggested that CNI-1493 can broadly block by-products of macrophage activation that may contribute significantly to the toxicity of IL-2 without interfering directly with lymphocyte activation by IL-2. In fact, in a rat hepatoma model, the use of CNI-1493 allowed the administration of IL-2 at doses 10-fold higher than the maximum tolerated dose and protected the animals from toxicity while enhancing the antitumor effects of IL-2 (70). Unfortunately, early clinical trials with this compound have failed to reproduce these preclinical findings. Although a reduction in IL-2-induced hepatic enzyme abnormalities was observed, other IL-2 related toxicities were unaffected by CNI-1493 (Atkins et al., manuscript in preparation).

Another potentially promising approach involves the use of agents that block NO production. NO produced by endothelial cells is one of the major regulators of vascular tone and blood pressure. Kilbourn and colleagues have shown that the NO synthase inhibitor N-methyl arginine was able to rapidly reverse the hypotension induced by high-dose IL-2 in patients with metastatic renal cell carcinoma (71). Additional clinical trials of this agent in combination with IL-2 are in progress.

2.5. Potential Correlates of Response and Resistance

Because of the toxicity of high-dose IL-2 and the fact that only a small minority of patients respond to IL-2-based immunotherapy, several investigators have attempted to identify factors predicting responsiveness to IL-2. As mentioned previously, in the 270 patient database presented to the FDA, tumor response was significantly associated with a baseline ECOG performance status of 0 and the absence of prior systemic therapy (37). There was no association of response with the number of metastatic sites or the amount of therapy administered. Likewise, in the NCI Surgery Branch database, achievement of complete response correlated with the absence of prior immunotherapy. In this analysis, the total dose of IL-2 administered and the degree of rebound lymphocytosis after cessation of IL-2 also correlated with response (40).

In a recent French multicenter study involving patients treated with either IL-2 alone (19 patients), IL-2 plus IFN α (29 patients) or cisplatin (CDDP)/IL-2/IFN α (33 patients), tumor response was shown to correlate inversely with pre-treatment levels of IL-6, cAMP-receptor protein (CRP), and lactate dehydrogenase (LDH) (72). In a multivariate analysis, a low baseline serum CRP level and disease confined to skin, subcutaneous tissue, or lymph nodes (Mla) were most predictive of response and survival. In another analysis of 65 patients treated with IL-2 and IFN α , high serum LDH, poor performance status, and large tumor burdens were associated with poor response to treatment and poor overall survival (73).

Several studies have documented correlations between tumor response and the development of vitiligo (74) and other autoimmune phenomenon such as thyroid dysfunction (75). Vitiligo has been the most extensively studied and, in one recent study, was observed in 11 of 74 patients with melanoma who were followed for greater than 1 yr after receiving high-dose IL-2 (74). Of note was the fact that it was noted only in patients exhibiting an objective tumor response. None of 27 nonresponding patients with melanoma followed for 1 yr developed vitiligo ($p = 0.0002$). Vitiligo did not develop in patients receiving IL-2 for metastatic renal cell cancer, suggesting that a relative abundance of melanocyte-derived antigens might be a critical factor in the induction of specific T cell immunity to pigmented cells during IL-2 treatment.

Many groups have attempted to correlate responses to IL-2 treatment with HLA phenotype. The NCI Surgery Branch, for example, reported an association between tumor response and the HLA-A11 and A19 alleles, especially in patients receiving IL-2 with TIL (76,77). HLA-DR4 expression was associated with increased IL-2 toxicity (77). However, these associations were not as strong or convincing in subsequent analyses that included larger numbers of patients (78). Another study correlated HLA-DQ1 expression with tumor response ($p = 0.0017$) and survival ($p = 0.026$) (79). Finally, Scheibenbogen et al. noted that HLA-CW7 and HLA-A1 phenotypes were more frequent in patients who responded to IL-2 and IFN (80). In general, it has been difficult to confirm the correlations between these various HLA phenotypes and either antitumor response or the severity of side effects. As a consequence, the use of HLA phenotype as an eligibility criterion for access to IL-2-based immunotherapy does not appear justified.

Resistance to IL-2 therapy has been postulated to be due to a number of factors including the induction of apoptosis in activated tumor-infiltrating T cells as a result of tumor cell expression of Fas ligand (81) or T cell dysfunction due to diminished expression of the T cell antigen receptor ζ chain and other signaling components (82). Of note, Rabinowich et al. have shown that these T cell receptor abnormalities can be reversed by IL-2 immunotherapy and that such reversal may correlate with tumor response (83). Additional, correlative laboratory studies performed in conjunction with current and future clinical trials will be necessary to sort out the importance of these various molecular phenomena to the antitumor effects of IL-2.

3. IL-2 IN COMBINATION WITH OTHER BIOLOGIC RESPONSE MODIFIERS

3.1. IL-2 in Combination with IFN α

The decision to investigate the antitumor effects of IL-2/IFN α combinations is based on an abundance of persuasive preclinical laboratory and animal studies (84–87). Investigations involving various combinations of IL-2 and IFN α are summarized in Table 3. In one of the first dose escalation phase I/II clinical trials assessing the combination intravenous bolus IL-2 at 2.6–12 MIU/m² per dose and intravenous IFN α at 3–6 MU/m² per dose (Hoffmann-LaRoche) every 8 h on d 1–5 and 15–19 was administered to 91 patients, 39 of whom had metastatic melanoma (88). Tumor responses were seen in 25 patients (7 CR, 18 PR) including in 13 patients with metastatic melanoma (3 CR, 10 PR). The response rate was as high as 41% among the 27 patients treated at the highest doses of IL-2 (12 MIU/m²) and IFN α (6 MU). Toxicity was similar to that seen with high-dose IL-2 alone regimens, except for a higher than expected incidence of cardiac and neurologic toxicity. In 1995, Marincola and colleagues published long-term fol-

Table 3
IL-2 and IFN α Containing Treatment Regimens

<i>IL-2 Administration route/dose level</i>	<i>N</i>	<i>CR/PR</i>	<i>Response rate (%)</i>
High-dose bolus	217	12/36	22
Continuous infusion	259	6/33	15
Subcutaneous injection	51	0/3	6

Adapted from Atkins, Shet, and Sosman (67).

low-up data of the NCI Surgery Branch experience using the combination of IL-2 and IFN α . This update included data from 189 patients, of which 82 had melanoma (89). The response rate was 24% (20/82) in the patients with melanoma. However, of the 20 patients with melanoma that responded to the IL-2/IFN combination, only one was a long-term progression-free survivor. The investigators concluded that this combined regimen provided no obvious benefit relative to high-dose bolus IL-2 alone.

The Cytokine Working Group (CWG) performed a randomized phase III comparison of intravenous bolus IL-2 with or without IFN α . In this study, IL-2 was administered 3 times daily for 5 d, either alone at 6 MU/m²/dose (16 MIU/m²) or at 4.5 MU/m²/dose (12.6 MIU/m²) in combination with IFN α at 3 MU/m²/dose (90). As with the NCI Surgery Branch study, both the IL-2 and IFN α were supplied by Hoffmann-LaRoche. The toxicities of these regimens were equivalent. Eighty-five patients were enrolled, with 44 being randomized to IL-2 alone and 41 to IL-2 plus IFN α , respectively. Responses were seen in only 6 patients—2 of 44 (5%) patients receiving IL-2 alone and 4 of 41 (10%) patients receiving both IL-2 and IFN α . There were no complete responses on either arm. The study was terminated early due to the disappointing results on both arms. However, as with the other trials, it was clear that the addition of IFN α failed to result in a meaningful improvement over standard regimens involving high-dose IL-2 alone.

Two alternative IL-2/IFN regimens have also been explored. Patients received IFN α 10 MU/m²/d subcutaneously for 5 d, followed by IL-2 administered either by a 5-d continuous infusion at 18 MIU/m²/d or in accordance with a “decrecendo” schedule, in which IL-2 was administered at 18 MIU/m² over 6 h, followed by 18 MIU/m² over 12 h, 18 MIU/m² over 24 h, and finally a maintenance dose of 4.5 MIU/m²/d for 72 h (91). This decrescendo regimen was designed both to induce optimal IL-2 receptor expression and to minimize toxicity by avoiding the induction of high circulating levels of secondary cytokines. The 18 MIU/m²/d IL-2 schedule produced 5 responses in 27 patients (1 CR and 4 PR; 18% RR; 6–36%; 95% CI), while the decrescendo IL-2 schedule yielded 11 responses in 27 patients (3 CR, 8 PR; 41% RR; 22–61%; 95% CI) and an impressive 17-mo median survival. Twenty-two percent of patients whose disease had progressed follow-

ing dacarbazine (DTIC) chemotherapy responded to the decrescendo IL-2/IFN regimen. In addition to this encouraging antitumor activity, this regimen had a more favorable toxicity profile than the more conventional continuous infusion schedule. The European Organization for Research and Treatment of Cancer (EORTC) Melanoma Cooperative Group recently conducted a large-scale phase III randomized trial assessing the effect of adding cisplatin to the aforementioned decrescendo IL-2/IFN α regimen (92). Of the 66 patients assigned to the IL-2/IFN α alone arm, 18% responded (4 CR and 8 PR). Although this response rate was less than that of the cisplatin/IL-2/IFN α combination arm, overall and progression-free survival were equivalent for both limbs of the study.

Investigators at M.D. Anderson recently evaluated the effects of adding IFN α to their previously described continuous infusion IL-2 regimen. They observed only 2 PR out of 23 patients (8% RR; 1–28%; 95% CI) (93). In a large phase II multicenter Netherlands-based trial, 57 patients with melanoma were treated with IL-2 at 7.8 MIU/m²/d (Hoffmann-LaRoche) by continuous infusion and IFN α at 6 MU administered subcutaneously on d 1–4 every 2 wk. Responses were seen in 16% of the 51 evaluable patients (8 responses; 1 CR, 7 PR) (94). A similar regimen utilizing IL-2 (Hoffmann-LaRoche) at 5.2 MIU/m²/d plus IFN α was explored with no responses noted in 14 melanoma patients (95). Likewise, no responses occurred among 15 patients with melanoma treated with a similar regimen, in which IL-2 was administered by subcutaneous injection (96).

Finally, 3 times weekly, IFN α with low-dose IL-2 (60,000 IU/kg) was administered as an intravenous bolus injection every 8 h for up to 14 doses on d 1–5 and 15–19 (97). Among 38 evaluable patients, 6 responses were observed with 1 CR and 5 PR (15% RR; 4–27%; 95% CI). Three of the 6 responses were over 12 mo in duration, with 2 ongoing at 26+ and 40+ mo.

Despite promising preclinical data, there is presently no convincing evidence suggesting that IL-2 and IFN α combinations are inherently superior to high-dose IL-2 alone. Nonetheless, because some IL-2 and IFN α combinations have demonstrated an improved therapeutic index relative to high-dose IL-2, this combination has provided a more suitable building block for clinical trials using IL-2 in combination with other biologic agents or cytotoxic chemotherapy.

3.2. IL-2 in Combination with Other Immune Activating Agents

A variety of clinical trials evaluating IL-2 in combination with immune modulators other than IFN α have been performed, and the results of these studies are described in Table 4. A few trials have examined combinations of IFN γ and IL-2. IFN γ activates macrophages and granulocytes and enhances MHC class I and II and tumor antigen expression. The NCI Surgery Branch evaluated the antitumor effects of IFN γ administered prior to standard high-dose intravenous bolus IL-2 (98). Some of the study patients also received TIL. Only 2 of the 20 patients enrolled in this study (and none of the 5 receiving TIL) responded.

Table 4
IL-2 with Other Immune Activating Agents

Author date	Reference	Interleukin-2			Other agent	Melanoma	Results		
		Dose	Route	Response					
				CR			PR	RR (%)	
Viens et al. 1992	99	8 MIU/m ²	IVBQ8H	IFN γ 1 $\times$ 10 ⁶ IU/m ² escalating doses	11	1	1	8%	
Thatcher et al. 1990	109	11 MIU/m ²	IVQOD ^a	FAA 4.8 gm/m ²	34	1	4	15%	
O'Reilly et al. 1993	110	6–18 MIU/m ² /d	IV CI	FAA 4.8 gm/m ²	21	1	2	14%	
Kim et al. 1995	98	720,000 IU/kg	IVBQ8H	IFN γ	20	1	1	10%	
				IFN γ plus TIL	5	0	0		
Ahmed et al. 1996	107	3 MIU/m ² /d	SQ	Levamisole 50 mg tid	18		1	12%	
		5.4 MIU/m ² /d	SQ	Levamisole 50 mg tid	21		1	12%	
		escalating doses		with or without					
Olencki et al. 1996	101	3, 12, 48 MIU/m ²	IV 3 times/wk	IL-4	39	0	2	5%	
				40, 120, 400 μ g/m ²					
Creagan et al. 1997	104	3 MIU/m ² /d	SQ	Levamisole 50 mg/m ² tid PO	19	0	0	0	

^aAdapted from Atkins, Shet, and Sosman (67). IU, international units; MIU, million international units; IVBQ8H, intravenous bolus every 8 h; IVQOD, intravenous every other day; SQ, subcutaneous; CR, complete response; PR, partial response; RR, response rate.

Other phase I trials assessing IFN γ combined with lower dose, more protracted, IL-2 schedules in patients with melanoma were equally disappointing (99,100).

Several investigators have explored IL-2 in combination with IL-4 (101,102). The rationale for this combination is the known ability of IL-4 to enhance tumor-specific T cell responses and nonspecific NK/LAK cell-mediated cytotoxicity. Thus far, studies with this cytokine combination have been disappointing, in that only a few tumor responses and minimal enhancement of the immunomodulatory effects of IL-2 have been noted. While IL-4 continues to be of interest as a growth factor to be used in combination with GM-CSF to propagate ex vivo dendritic cells for use in vitro or in vaccine development (103), studies involving IL-4/IL-2 combinations as treatment for overt metastatic malignancy have not been further pursued.

The Mayo Clinic has investigated a combination of IL-2 with levamisole, an antiparasitic agent purported to have immunostimulatory properties (104). Levamisole has shown potential benefit as an adjuvant to surgical resection in patients with high-risk melanoma (105,106). Unfortunately, a phase II trial of subcutaneous IL-2 and levamisole demonstrated no objective responses in 19 melanoma patients treated with this combination (104). In a similar study carried out at the Royal Marsden Hospital by Ahmed et al. using subcutaneous IL-2 at 3 and 5.4 MIU/m²/d with or without levamisole, responses were seen only in those patients receiving IL-2 without levamisole (107), suggesting that the combination offers little advantage over IL-2 alone.

Flavone-8-acetic acid (FAA) activates NK cell activity and, when given with IL-2, exerts synergistic antitumor effects in animal tumor models (108). Based on these preclinical data, Thatcher and colleagues performed a phase II trial of IL-2 in combination with FAA in patients with metastatic melanoma (109). In this study, IL-2 was first administered via a femoral catheter intrasplenically and then by intravenous bolus on alternate days while FAA was administered intravenously as a 6-h infusion prior to the IL-2. Five responses, including 1 CR, were observed among the 34 study patients. Responses occurred mainly in nonvisceral sites. The authors felt that this response rate was not significantly better than IL-2 alone. O'Reilly explored the use of FAA in combination with continuous infusion IL-2 and observed 3 responses (1 CR, 2 PR) in the 21 study patients (overall response rate of 14%), again suggesting no advantage over IL-2 alone (110).

Several investigators have recently become interested in the combination of IL-2 with histamine (111). This biogenic amine can inhibit monocyte-derived oxygen free radical formation. Some investigators believe that monocyte free radical formation inhibits T cell and NK cell tumoricidal activity, hence the rationale for the combination. A randomized trial of IL-2 plus histamine versus IL-2 alone, in patients with metastatic melanoma, has recently been completed and is pending analysis.

IL-2 has been shown to enhance IL-12 receptor expression and IL-12-induced IFN γ production, suggesting that these two cytokines might act synergistically to eradicate tumor cells (112, 113). Indeed, Wiggington et al. have shown that the combination of IL-2 with IL-12 has synergistic antitumor activity in a murine renal cell carcinoma model (114). Phase I trials to evaluate this combination in humans have recently opened at the NCI and Beth Israel Deaconess Medical Center in Boston.

Although some of these approaches with combinations of immune modulating agents appear promising, one must conclude that at the moment, no convincing evidence exists that any immunologic agent can add to the antitumor activity of IL-2 alone in metastatic melanoma.

3.3. *IL-2 and Adoptive Immunotherapy*

The initial reports of effective IL-2 treatment demonstrated that the combination of high-dose IL-2 with ex vivo IL-2-activated PBMCs (LAK cells) had activity against various cancers (115, 116). PBMCs were obtained via lymphocytapheresis performed during the rebound lymphocytosis following a 5-d cycle of high-dose IL-2. PBMCs were subsequently activated to create LAK cells by exposure to IL-2 in bags or flasks and reinfused with another cycle of high-dose IL-2. Eleven out of the first 25 patients had objective tumor responses, with most responses occurring in patients with either kidney cancer or melanoma (115). These results were expanded upon in a multiple subsequent reports (116).

The promising results from the initial NCI Surgery Branch studies led other groups to explore the combination of IL-2 and LAK cells (Table 5). In the phase II experience of the 6-member IL-2 Extramural Working Group (IL2-EWG) with high-dose bolus or continuous infusion IL-2 plus LAK cells (35, 117), there were 6 responses among 32 patients (19%) in the bolus IL-2/LAK cell trial, while only 1 response among 33 patients (3%) was observed with the continuous infusion IL-2/LAK regimen. The IL2-EWG experience with a combined regimen utilizing bolus IL-2 on d 1–3 and continuous infusion IL-2 with periodic LAK cell infusions on d 10–14 produced 7 responses (1 CR, 6 PR) for a 14% RR in 50 evaluable patients (118). The NBSG reported a long-term follow-up on patients receiving continuous infusion IL-2 together with LAK cells. They observed only 4 responses among 33 melanoma patients (12%) with a median survival of only 6.1 mo (119). Other lower dose IL-2 plus LAK cell regimens have been uniformly disappointing with very few objective responses in melanoma patients (120). In a randomized trial using weekly continuous infusion IL-2 versus IL-2 plus LAK, Koretz et al. observed no objective responses in 18 patients with melanoma despite significant in vivo stimulation of LAK and NK cells (121).

The NCI Surgery Branch performed a phase III trial of high-dose intravenous bolus IL-2 with or without LAK cells (122). Fifty-four of the 181 patients entered

into the trial had melanoma. Overall, the survival and response rate was not significantly improved for patients receiving IL-2/LAK treatment; however, survival in melanoma patients was slightly prolonged. Survival rates of 32% vs 15% and 18% vs 4% were observed at 24 and 48 mo for patients treated with IL-2/LAK cells and IL-2 alone, respectively. Nonetheless, because of the general impracticality of the LAK cell generation procedure and the absence of a compelling therapeutic benefit, LAK cell therapy has been abandoned as an adjunct to IL-2.

Subsequently, Rosenberg and his colleagues explored the use of more specific CTLs generated from TILs (123). Animal studies had demonstrated TILs to be greater than fiftyfold more effective in their antitumor activity than LAK cells (28). The initial report of autologous TILs plus IL-2 was very promising, with over a 40% RR in patients with melanoma (124). A larger cumulative experience, including 86 patients with metastatic melanoma treated with TILs and high-dose IL-2, showed a 34% RR (125). Responses were observed as frequently in patients who had failed high-dose IL-2 as those who were previously untreated with IL-2. Most of these responses were partial, however, and of short duration. Considering that this treatment was primarily limited to patients with relatively slow growing disease and biopsy-accessible tumor, which contained expandable TILs, it was uncertain to what extent these apparently improved results relative to high-dose IL-2 alone were a consequence of selection bias.

Numerous other smaller studies have explored the application of TILs plus IL-2 with varying results. Tumor responses have been observed, and at times, these clinical responses have correlated with in vitro cytotoxicity against autologous tumor cells. Thirty-seven consecutive patients (28 with measurable disease after excisional biopsy for TIL isolation) were treated with TIL infusions ($>10^{10}$ cells) and low-dose IL-2 (4.5 MIU subcutaneously 3 times daily for 3 d) (126). Eight of 28 patients had tumor regression (3 CR, 2 PR, 3 minor response [MR]). Further analysis revealed that there was a significant correlation between the level of CD8⁺ cells after IL-2-TIL therapy and both tumor response and overall survival. Median overall survival was 12 mo (range 1–54 mo). After freshly isolating TIL from stage IV melanoma patients, Arienti and colleagues cultured these cells in low-dose IL-2 and then high-dose IL-2 (127). Sixteen patients subsequently received TIL (mean 6.8×10^9 cells) and IL-2 (mean dose 130 MIU; range 28.8–231 MIU). There was 1 CR and 3 PR in 12 evaluable patients. Infused TIL showed an preferential lysis of autologous tumor cells in vitro in all the responding patients. Finally, TIL and IL-2 combinations have been examined in the adjuvant setting with some encouraging results (128).

Other investigators have attempted to activate specific lymphoid effector cell populations using strategies that did not require the harvesting and activation of TIL (129,130). Chang and his colleagues obtained lymphocytes from draining lymph nodes of patients vaccinated with an autologous tumor plus Bacille

Table 5
IL-2 Combined with Adoptive Cellular Therapy

Author year (ref.)	IL-2 Dose	Route	Additional treatment	Patients (total)	Response		Total %	Comments
					CR	PR		
High-dose bolus IL-2								
Dutcher et al. 1989 (35)	600,000 IU/kg	IV	LAK cell infusion, on d 12, 13, and 15.	32	1	5	19	Priming dose for 5 d. 8 th day leukapheresis, followed by another 5 d IL-2 course.
Bar et al. 1990 (118)	600,000 IU/kg 3 MIU/m ²	IV bolus IV CI for 148 h	LAK cell infusion, on d 9, 10, and 12.	50	1	6	14	Priming dose for first 3 d, followed by CI for 148 h on d 9-15. Total 15-d cycle.
Rosenberg et al. 1993 (122)	720,000 IU/kg	IV Q8H	LAK cell infusion, on d 11, 12, and 14.	(54)	0	6	22	Randomized control trial: IL-2 vs IL-2 plus LAK.
Rosenberg et al. 1994 (124)	720,000 IU/kg	IV Q8H	TIL plus CTX 25 mg/kg × 1 dose	22 86	3 5	3 24	27 34	No. of pts reported reflects the no. in whom TILs were successfully harvested.
High-dose continuous IV infusion IL-2								
Dillman et al. 1991 (119)	18 MIU/m ² /d	IV CI × 5d IV CI × 5d	LAK cell infusion, on 11, 12, and 13.	53	CR + PR =	12	23	Combined results of studies by NBSG.
Dutcher et al. 1991 (117)	18 MIU/m ² /d 22.5 MIU/d	IV CI × 5d IV CI × 5d	LAK cell infusion, on d 11, 12, and 14	33	0	1	3	CWG results using IL-2 CIV.
Arienti et al. 1993 (127)	28.8-231 MIU/d	IV CI	TIL infusion no. of cells = 6.8 × 10 ⁹	16	1	3	33	Mean dose of IL-2 = 130 × 10 ⁶ IU/d Mean no. of TIL = 6.8 × 10 ⁹ cells.

Moderate-dose IL-2

Chang et al. 1997 (130)	3.6 MIU/kg	IVBQ8HR	OKT3 and IL-2-activated TIL	23	0	1	6	TIL harvested from draining lymph nodes after vaccination and activated with IL-2 and OKT3 in vitro. 3 minor responses not included in results. Escalating dose of IL-2 1.5×10^6 IU/d. Day 0-5
Calvo et al. 1997 (126)	4.5 MIU	SC	TIL	28	3	2	22	
Reali et al. 1977 (128)	3-9 $\times 10^6$ IU	SC	TIL plus IFN α	24	—	—	—	
Curti et al. 1998 (129)	9 MIU/d	IV CI	CD4 cell infusion CTX 1000 mg/m ²	14	0	1	8	
Koretz et al. 1989 (121)	3 MU/m ² /d	IV CI	LAK infusion	18	0	0	0	Randomized trial of IL-2 vs IL-2 plus LAK therapy.

CR, complete response; PR, partial response; IU, international units; MIU, million international units; IV CI, intravenous continuous infusion; IVQ8H, intravenous every 8 h; TIL plus CTX, tumor infiltrating lymphocytes; OKT3, anti-CD3 antibody; SC, subcutaneous.

Calmette-Guérin (BCG) vaccine (130). These lymphocytes were activated *in vitro* with anti-CD3 and IL-2 and reinfused along with additional IL-2. The T cells expanded this way had minimal cytotoxicity for autologous tumor, but exhibited highly specific cytokine release (IFN γ and GM-CSF). There was one PR observed in 11 patients with melanoma. Curti et al. at the NCI-Biologic Response Modifiers Program (BRMP) explored the merits of CD4-selected T cells activated *ex vivo* with anti-CD3 and IL-2 (129). These CD4⁺ T cells were then infused with additional systemic IL-2. CD4⁺ cells were expanded *in vivo* following infusion, and cells appeared to traffic to sites of subcutaneous melanoma deposits. Tumor responses were observed in 3 of 31 patients (1 CR, 2 PR). The responses were seen in patients receiving larger numbers of CD4⁺ T cells. Despite all of the above efforts, and some encouraging results, the true value of TIL and other adoptive immunotherapy approaches in combination with IL-2 relative to IL-2 alone remains to be determined. Nonetheless, the tremendous labor and cell preparation costs involved with this treatment approach have limited or even abrogated its use in future clinical investigations.

3.4. IL-2 and Monoclonal Antibodies

The prospect of combining IL-2 with monoclonal antibodies (MAbs) in the treatment of melanoma is based on several studies that suggest that the two agents may interact in a synergistic fashion to eradicate tumor cells. Antibodies reactive with tumor cells can bind to unique tumor antigens, rendering the tumor cells susceptible to clearance by activated Fc receptor-positive phagocytes and NK cells. Indeed, IL-2 has been repeatedly shown to enhance ADCC (44,45,131–134). Alternatively, antibodies directed at molecules on the surface of the host effector cells (e.g., CD3, CD2, CD16) activate these cells and might render them more receptive to IL-2-mediated expansion (135–138). Recent advances in biotechnology have made possible the creation of bispecific MAbs capable of simultaneously binding to both effector cells and tumor cells, thereby facilitating the formation of effector:target conjugates and the lysis of the tumor target cell. Similar techniques have led to the production of antibody:cytokine conjugates, which bind to tumor cells and activate proximal immune effector cells, expressing receptors for the particular cytokine (139,140).

Much of this research focused on ganglioside-specific MAbs. Gangliosides are glycolipid molecules composed of a sialylated oligosaccharide chain linked to a ceramide fatty acid core. Melanoma cells are rich in gangliosides, two of which (GD2 and GD3) appear to be up-regulated by transformation of melanocytes (141,142). Gangliosides are also present on the surface of certain T cells, where they are able to trigger activation signals when engaged by a specific antibody (135). Phase I clinical trials of unconjugated ganglioside-specific MAbs have shown that they can reach tumor sites after systemic administration. Some

tumor responses have been observed with R24, (anti-GD3), 3F8 (anti-GD2), and other murine MAb (*142–147*).

The anti-GD3 antibody R24 has been the MAb most actively investigated in combination with IL-2. In the initial study with this antibody at the Memorial Sloan Kettering Cancer Center, IL-2 was administered at 6 MIU/m² intravenously over 6 h on d 1–5 and 8–12, while R24 was given at one of 4 dose levels ranging between 1 and 12 mg/m² daily on d 8–12 (*148*). The extent of T cell activation and the magnitude of the rebound lymphocytosis at the completion of the IL-2 treatment correlated with the R24 dose. Of the 20 treated patients, 1 PR and 2 MR were noted. The R24 MAb was combined with very low-dose continuous infusion IL-2 administered over a course of 8 wk with the antibody given during wk 5 and 6 (*149*). Of the 28 patients with metastatic melanoma enrolled, only 1 PR (regression of liver metastases from a primary ocular melanoma) was observed. Alpaugh et al. recently reported the results of a phase IB trial that utilized a 5-d continuous infusion of R24 followed by 3 wk of subcutaneous IL-2 and IFN α (*150*). Twenty melanoma patients were enrolled. Tumor biopsies obtained prior to treatment, on d 8 (prior to the initiation of IL-2 and IFN α) and on d 29 revealed progressively increasing chronic inflammation; however, it was difficult to determine to what extent the R24 may have contributed to this inflammatory reaction. In any event, there were no objective responses seen in the 18 evaluable patients.

The administration of murine antibodies to humans inevitably leads to the production of neutralizing human antimouse antibody (HAMA). This limitation, as well as the relatively poor interaction between murine antibodies and human Fc receptor positive immune effectors, has led to the generation of “humanized” antibodies with longer half-lives and better ADCC activation. In a phase I clinical trial evaluating one such antibody, IL-2 was continuously infused along with a chimeric anti-GD2 ganglioside antibody (14.18) (*151*) constructed by ligating DNA sequences encoding the Fab fragment of the mouse 3F8 MAb into sequences specifying a human immunoglobulin molecule. The 14.18 antibody/IL-2 combination enhanced ADCC and LAK activity and reduced human anti-chimera antibody (HACA) formation. Antitumor activity was observed in 2 of the 24 study patients (1 CR, 1 PR). One case of dose-limiting peripheral neuropathy was observed. Concomitant laboratory studies suggested that the addition of IL-2 might have suppressed anti-idiotypic antibody formation induced by the 14.18 chimera (*152*).

Although some tumor responses have been noted in trials assessing combinations of IL-2 with antiganglioside antibodies, they are sufficiently rare so as to dissuade further clinical investigation in patients with metastatic melanoma at the present time. More potent MAb and additional data on how to optimally combine MAb with IL-2 are prerequisites for further investigation of this approach to immunotherapy.

The use of IL-2 in combination with MABs directed against T cell antigens has also been explored. Preclinical studies support the concept that anti-CD3 MAB administered at low doses can activate T cells to release cytokines and proliferate and mediate antitumor effects (*136–138,153*). Anti-CD3 MAB OKT3 in doses ranging from 10–600 µg was used in conjunction with IL-2 administered either by continuous low-dose intravenous infusion, subcutaneous injection, or high-dose intravenous bolus (*154–157*). These trials failed to demonstrate the induction of CD25, a component of the high-affinity IL-2R on circulating T cells despite the fact that this activation antigen is readily induced by OKT3 in vitro. Furthermore, only one objective response was observed among 16 patients with melanoma treated with OKT3 and IL-2 (*157*). However, it is possible that this strategy of combining IL-2 with T cell-specific MAB might be more effective if bispecific MABs or fusion antibodies, which are able to activate effector cells directly at the tumor cell surface, were to be used.

In an effort to enhance ADCC at the tumor site, IL-2 was linked to the carboxy-terminus of the aforementioned chimeric 14.18 molecule (*158*). This fusion protein binds to GD2-expressing tumor cells in vitro as avidly as the unmodified 14.18 antibody and mediates ADCC against GD2-expressing melanoma cells by stimulating both cytotoxic T cells and NK cells expressing IL-2Rs, regardless of whether they express Fc receptors. Animal models using this fusion protein have shown it to be superior to either IL-2 or 14.18 alone or both in combination at comparable stoichiometry (*139,159,160*). Phase I trials of this fusion protein in melanoma patients have recently been initiated.

3.5. IL-2 and Tumor Vaccines

Melanomas possess tumor antigens that can be recognized by the host immune system (*161*). Over the past several decades, numerous investigators have attempted to exploit this attribute to develop specific vaccines capable of inducing T cell-mediated tumor rejection. Most of these early attempts made use of crude melanoma cell preparations. While some of these approaches showed initial promise, beneficial effects have yet to be confirmed in phase III trials (*161–167*). Efforts to develop an effective vaccine have been hindered by poor immunogenicity of certain antigens, tumor-induced tolerance, antigen shedding, and heterogeneity with respect to tumor cell antigen expression. Since IL-2 is able to induce the clonal expansion of antigen-primed T cells, it follows that IL-2 would be a useful adjunct to any vaccine regimen under development. As a consequence, several investigators have included IL-2 as a key component in their various tumor vaccine protocols. A summary of recent trials, pairing IL-2 with some sort of tumor vaccine, is displayed in Table 6.

An allogenic liposomal melanoma vaccine has been administered with or without systemic or regional low-dose IL-2 (*168*). Nine of the 24 vaccinated patients had objective responses, including 6 responses (3 CR, 3 PR) in the 10 patients

Table 6
IL-2 Combined with Vaccines

<i>Author year (ref.)</i>	<i>Interleukin-2</i>			<i>Schedule</i>	<i>Type of vaccine</i>	<i>Schedule</i>	<i>N</i>	<i>Response</i>		<i>Total response %</i>
	<i>Dose</i>	<i>Route</i>	<i>Schedule</i>					<i>CR</i>	<i>PR</i>	
1. Adler et al. 1995 (168)	Variable bolus	Intravenous or regional	Q8H	Allogenic liposomal melanoma vaccine	SQ injection at 2 sites every week × 10 wk	24 ^a	3	6		50% ^a
2. Oratz et al. 1996 (169)	0–5 × 10 ⁵ IU	Liposome encapsulated d IL-2		Polyvalent melanoma vaccine encapsulated in liposomal IL-2	ID Q2 wk × 4 and monthly × 3	8 ^b	1	1		25%
3. Rosenberg et al. 1998 (180)	720,000 IU/kg	IVB	Q8 H × 5 d for 2 cycles	Modified gp100 peptide vaccine (G209–2M)	1.0 mg SQ into thigh. 2–6 Immunizations at 3-wk intervals	31	1	12		42%

^aStudy included 4 arms of which responses were seen in the IL-2 plus vaccine arm and the regionally administered IL-2 arm only.

^bOnly 8 patients had measurable disease of the 36 in the group studied.

CR, complete response; PR, partial response; SQ, subcutaneous; ID, intradermal; IVB, intravenous bolus; IU, international units.

who received the vaccine plus low-dose regional IL-2. Unfortunately, neither follow-up reports on these patients nor additional trials confirming these remarkable results have been forthcoming. Oratz and colleagues have evaluated a polyvalent vaccine, in which shed melanoma antigens and IL-2 were both encapsulated into liposomes in an effort to increase vaccine immunogenicity (169). Thirty-six patients with stage IV melanoma received this vaccine, followed by daily low-dose IL-2 injections ($2-5 \times 10^5$ IU) for 2 wk. This regimen was felt to induce a higher frequency and duration of vaccine-induced delayed-type hypersensitivity (DTH) responses relative to historical controls receiving vaccine alone, an effect attributed to the addition of IL-2 (169). Of 28 patients whose metastatic disease had been completely resected at entry, 9 of 12 that had been followed for 2 yr and remained alive compared with only 15–30% of “matched” historical controls. There were 2 tumor responses (1 CR, 1 PR) observed in the 8 patients with measurable disease. Longer follow-up and randomized controlled studies are clearly necessary in order to determine the true value of this combination.

The recent identification and characterization of melanoma antigens specifically recognized by cytotoxic T cells has led to a shift in vaccine development towards the use of defined peptides rather than crude tumor cell lysates as a source of antigen (170–172). These antigens include embryonic proteins (MAGE family), as well as tissue-restricted (melanocyte differentiation) proteins such as tyrosinase, MART-1, and gp100 (173–179). Rosenberg and colleagues have investigated the immunogenicity of an 8-amino acid peptide derived from the gp100 protein (residues 209–217) and a mutated version of this peptide (gp100 peptide 209-2M) which binds HLA-A2.1 more effectively than the wild-type peptide (180). While the mutated peptide (gp100:209-2M) administered in incomplete Freund’s adjuvant alone was quite effective in inducing specific T cell immune responses (10/11 patients), few antitumor effects were observed. However, 13 (42%) of 31 patients who received the peptide together with high-dose IL-2 had an objective tumor response. A confirmatory trial of this combination is currently underway within the CWG and a phase III trial randomizing patients to either high-dose IL-2 alone or in combination with the gp100:209-2M peptide is being contemplated.

4. IL-2 GENE THERAPY

As previously discussed, IL-2 administered systemically enhances the immunogenicity of dendritic cell-based vaccines (24). In an effort to develop melanoma vaccine protocols that take advantage of the proliferative effects of IL-2 on activated T cells, but avoid the toxicity associated with the systemic administration of the cytokine, several investigators have pioneered vaccine regimens utilizing tumor cells genetically engineered to secrete IL-2 or mixtures

of tumor cells with IL-2-secreting fibroblasts. For example, murine melanoma cells engineered to secrete IL-2 elicited a greater degree of T cell activation in the lymph nodes draining the vaccination site than could be achieved with the parental untransfected tumor cells (181). In a small clinical trial in which 12 melanoma patients were vaccinated with one of two IL-2-transduced human melanoma cell lines, the investigators observed disease stabilization in 4 of the 12 patients treated (182). Likewise, in a clinical trial using autologous melanoma cells co-suspended with an allogenic immortalized fibroblast line engineered to secrete IL-2, vaccinated patients developed inflammatory reactions at the vaccination site, but little systemic toxicity. The DTH reactions at the vaccination site were characterized histologically by dense infiltrates of both CD4⁺ and CD8⁺ T cells. CD8⁺ T cells isolated from the vaccine sites and propagated *ex vivo* were cytolytic for autologous tumor cells (183). These results suggest that the high local concentrations of IL-2 achieved at the vaccine site by the aforementioned genetic manipulations might prove superior to the high circulating levels achieved by systemic administration as a means of enhancing the T cell response to tumor cell-based vaccines.

5. IL-2-BASED BIOCHEMOTHERAPY

Many investigators have studied combinations of IL-2-based immunotherapy and cytotoxic chemotherapy in this disease. Combinations including cisplatin and IL-2 have been the most promising, producing response rates consistently higher than those observed with either IL-2 or chemotherapy alone (184,185). It is, however, unclear if these combinations increase the number of patients surviving long-term disease-free. Multiple phase III trials comparing biochemotherapy to either chemotherapy alone or IL-2-based immunotherapy in patients with metastatic melanoma have been initiated. These trials should definitively address the value of biochemotherapy in this disease. This information is discussed in more detail in Chapter 10.

6. CONCLUSIONS

Modest progress has been made in the treatment of metastatic melanoma over the past decade. With the advent of high-dose IL-2, it is now possible to talk about “cure”, albeit in a small minority of patients. Abundant preclinical data support the use of IL-2 in combination with other cytokines, toxicity blocking agents, monoclonal antibodies, adoptive immunotherapy, or vaccines, and many of these approaches have yet to fully tested clinically. The wealth of promising new treatment strategies offers hope that “cure” for more than the rare patient with metastatic melanoma will be achieved within the coming generation. It is likely that IL-2 will be a critical component of whichever new approach gains ascendancy in the future.

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