Cytokines (IL-2, IFN, GM-CSF, etc.) Melanoma

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Contents

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Abstract

Treatment of advanced stage melanoma has recently undergone a revolutionary change. Prior to 2011 dacarbazine and interleukin-2 were the only treatment options with both demonstrating no impact on overall survival. However, the experience with and results with IL-2 therapy have stimulated development of novel immunotherapies in this disease. IL-2 was the first immunotherapy to be approved for patients with metastatic melanoma and produced durable complete remissions in about 6% of patients. The accompanying toxicity, however, limited the widespread use of IL-2 therapy. In contrast, interferon-alpha despite its important role in the adjuvant setting, never showed a significant response rate or any survival benefit in patients with stage IV disease.

Currently, due to the highly successful revival of immunotherapy with the development of immune checkpoint inhibitors targeting CTLA4 and PD1 on T-lymphocytes, the exploration of cytokines such as IL-2, IFN-a and others, now in conjunction with immune checkpoint inhibition or adoptive cell therapy has been rejuvenated in this disease.

This chapter summarizes the development and use of cytokines as single agents or in combination in the treatment of patients with advanced metastatic melanoma.

Interleukin-2

Interleukin-2 (IL-2) was the first biologic therapy approved by the US Food and Drug Administration for the treatment of patients with metastatic melanoma. The approval in 1998 was based on the collective experience with high-dose IL-2 that resulted in clinical response rates of 16%, including complete responses in 6%, many of which were durable (Atkins et al. 1999, 2000). Similar response rates were noted in patients with metastatic renal cell carcinoma, the only other cancer type for which high-dose IL-2 is currently approved (Fyfe et al. 1996).

IL-2 is a hormone produced by lymphocytes and was first described as a T cell growth factor in 1976 (Morgan et al. 1976). The first clinical use was in 1983 when Bindon and colleagues treated two patients with melanoma with natural IL-2, derived from stimulated normal lymphocytes (Bindon et al. 1983). Lotze and colleagues treated AIDS and cancer patients with IL-2 that was derived from Jurkat, a human T cell line (Lotze et al. 1984, 1985). Because of the limited availability of IL-2, small doses were used in these early studies and no anti-tumor responses were observed. These studies did however demonstrate that IL-2 was capable of modulating the immune system and was worthy of further study. The discovery of the gene for IL-2 (Taniguchi et al. 1983) and the production of recombinant IL-2 resulted in large quantities of IL-2 for clinical trials.

Initial studies evaluating different doses of administration of IL-2 defined the maximal tolerated intravenous dose to be 2.16×10^4 IU/kg/h when given by continuous infusion and 7.2×10^6 IU/kg by a single bolus injection (Lotze et al. 1985, 1986b). Because laboratory models predicted that tumor responses correlated with dose intensity (Papa et al. 1986), early studies by Rosenberg and colleagues utilized the highest tolerable intravenous bolus dose that could be repeatedly administered for 10–15 doses every 8 h (Rosenberg et al. 1985). This regimen of 600,000 or 720,000 IU/kg/dose given intravenously every 8 h became known as high-dose IL-2; with the 600,000 IU/kg/dose being the FDA approved regimen.

Pharmacology of IL-2

Currently the only commercially available IL-2 in the US is Proleukin® (Aldesleukin), produced initially by the Cetus Oncology Division of Chiron and now by Novartis, and licensed to Prometheus. Proleukin® varies from natural IL-2 in that it is non-glycosylated, the cysteine at amino acid position 125 has been replaced by serine and it lacks the N-terminal alanine. This recombinant IL-2 has been shown to be biologically and functionally similar to natural IL-2 (Rosenberg et al. 1984; Doyle et al. 1985). Following intravenous administration, IL-2 is rapidly cleared from the circulation according to a two-compartment model (Lotze et al. 1985; Konrad et al. 1990; Yang and Rosenberg 1997). The initial clearance (α distribution) has a half-life (t1/2) of 7–13 min consistent with a rapid egress of IL-2 into the extravascular space. Subsequently, a longer clearance (β clearance) with a t1/2 of 60–96 min is consistent with a slower release from the extravascular space back into the plasma compartment. Studies with subcutaneous IL-2 administration have shown lower peak serum levels than intravenous IL-2 and the levels remain fairly constant for 8–10 h (Yang and Rosenberg 1997; Konrad et al. 1990; Gustavson et al. 1989). Clearance of IL-2 from the body is primarily through metabolism in the kidneys.

Immunologic Activity of IL-2

Immunologic changes can be measured early following the systemic administration of IL-2. Lymphopenia develops within minutes of a single dose of IL-2 (Lotze et al. 1985; Thompson et al. 1988; Boldt et al. 1988; Punt et al. 1992; Sondel et al. 1988; Macfarlane et al. 1995). The lymphopenia is profound and persists while patients are receiving IL-2; it is thought to be secondary to margination and egress of lymphocytes into the extravascular space. Twenty-four to 36 h after discontinuation of IL-2, there is a rebound lymphocytosis of 2 to 70-fold above baseline which persists for 2–7 days; this rebound is dose and schedule dependent (Sosman et al. 1988; Macfarlane et al. 1995; Punt et al. 1992; Thompson et al. 1988).

Lymphokine activated killer (LAK) precursors and natural killer (NK) activity are lost from the circulation within 15 min of a single (bolus) dose of intravenous IL-2 (Lotze et al. 1985; Salvo et al. 1992). One to 2 days after discontinuation of IL-2 (bolus or continuous infusion), in vitro cytolysis of radiolabeled Daudi and K562 cell targets rebounds above baseline in a dose and schedule dependent fashion (Lotze et al. 1985; Thompson et al. 1988; Sosman et al. 1988; Gratama et al. 1993; Rosenthal et al. 1988). PBMC have decreased proliferative responses to IL-2, mitogens and soluble antigens during IL-2 therapy, and rebound after IL-2 discontinuation (Lotze et al. 1985; Rosenthal et al. 1988; Wiebke et al. 1988; Kradin et al. 1989). Repetitive IL-2 administration results in lymphocyte activation as demonstrated by the expression of the CD25 IL-2 receptor (Lotze et al. 1985, 1987; Thompson et al. 1988; Gratama et al. 1993) or HLA-DR expression (Thompson et al. 1988; Gratama et al. 1993) on the cell surface. Soluble IL-2 receptors (shed from the cell surface) are increased in the serum (Lotze et al. 1987; Urba

et al. 1990; Smith et al. 2003). At the tissue level, profound lymphocytic infiltrates have been documented in a variety of organs and tumors (Kragel et al. 1990; Rubin et al. 1989; Cohen et al. 1987). After IL-2 therapy, patients can develop immediate hypersensitivity (IH) to skin test recall antigens (Kradin et al. 1989), however, delayed type hypersensitivity (DTH) responses are depressed (Kradin et al. 1989; Wiebke et al. 1988).

B cell numbers decrease with IL-2 infusion (Wiebke et al. 1988; Thompson et al. 1987; Sondel et al. 1988) and return to baseline several days following discontinuation of IL-2; they do not rebound to the same extent as T cells (Wiebke et al. 1988). B cells products such as IgG, IgM, and IgA are moderately decreased during IL-2 infusion (Wiebke et al. 1988; Rosenthal et al. 1988; Kradin et al. 1989) and return to normal or higher after stopping IL-2. This phenomenon may be in part due to redistribution changes secondary to fluid shifts characteristic of the vascular leak syndrome typically seen with IL-2 administration (see below). Tetanus specific IgG is increased during IL-2 therapy following tetanus reimmunization, suggesting that B cell function is preserved (Rosenthal et al. 1988). Anti-IL-2 antibodies are frequent following intravenous IL-2 administration (Thompson et al. 1987; Allegretta et al. 1986; Sarna et al. 1994) and are generally non-neutralizing. Non-neutralizing antibodies have also developed after subcutaneous IL-2 administration (Atzpodien et al. 1990a). Neutralizing antibodies to IL-2 have been observed following subcutaneous administration of IL-2 with interferon β (Krigel et al. 1988) and in patients with renal cell cancer receiving subcutaneous IL-2 (Whitehead et al. 1990). Serum sickness has not been observed (Sarna et al. 1994; Atkins et al. 1986) and the clinical implications of anti-IL-2 antibody development are unknown.

Neutrophil counts have not been generally affected by IL-2. Severe neutropenia during or shortly after IL-2 therapy is rare (Sosman et al. 1988; Ettinghausen et al. 1987; Rosenberg et al. 1994). Neutrophils may be increased in the first 24 h of IL-2 along with a shift to immature forms (Macfarlane et al. 1995; Jablons et al. 1990). However, transient neutrophil dysfunction occurs as noted by decreased Fc receptor expression and decreased chemotaxis (Jablons et al. 1990; Klempner et al. 1990), and may contribute to an increased risk of catheter related bacterial infections seen with IL-2 therapy.

Many cytokines, including TNFα, IFNγ, GM-CSF, M-CSF, G-CSF, IL-5, IL-6, IL-8, and IL-10, have been shown to increase in the serum following IL-2 administration (Punt et al. 1992; Salvo et al. 1992; Konrad et al. 1992; Weidmann et al. 1992; Mier et al. 1988; Jablons et al. 1989; Gemlo et al. 1988; Tritarelli et al. 1991; Schaafsma et al. 1991; Tilg et al. 1993, 1995; van Haelst Pisani et al. 1991). This cytokine cascade has been implicated in many of the observed side effects of IL-2 administration.

Systemic Effects of IL-2

All major organ systems can be affected by IL-2 and much of the etiology of the side effects can be traced to a capillary leak syndrome (reviewed in (Schwartzentruber 2000)). The intensity of side effects is determined by the dose and schedule of IL-2 administration, but all regimens have the capacity of producing similar toxicities. For any given dose, continuous infusion IL-2 (over 24 h) is more toxic than the same dose given by bolus infusion (Thompson et al. 1988). Hence, the greatest dose intensity per cycle of treatment can be achieved by intermittent bolus dosing. The scant evidence of clinical benefit of low doses of IL-2 when used as a single agent and laboratory evidence in favor of dose intensity (Papa et al. 1986) have supported the continued use of the high-dose regimen. As experience has been gained, the incidence of side effects of high-dose IL-2 has diminished considerably (Kammula et al. 1998). It is clear that with careful patient selection and properly trained clinicians, the mortality from this therapy is extremely low (less than 1%) (Kammula et al. 1998). Another component of this improved safety profile is a decrease in the median number of IL-2 doses given per cycle from 13 to 8 at some centers, which has been

accomplished without apparent sacrifice in clinical activity (Kammula et al. 1998).

High-dose IL-2 (600,000 or 720,000 IU/kg) is given by intravenous bolus injection every 8 h. The treatment schema generally consists of two cycles of a maximum of 14 doses each, separated by a rest period of 10–14 days. Two cycles of treatment are generally referred to as one course; complete evaluation for clinical response is performed after every course or approximately every 2 months while on therapy. Re-treatment is offered at 2–3 month intervals to patients who show evidence of clinical response and is continued as long as the disease continues to diminish. Patients achieving a complete response generally receive one additional course of treatment after complete disappearance of disease if they are able to tolerate further IL-2. Ninety-one percent of patients with metastatic melanoma that responded to high-dose IL-2 achieved at least a partial response after one course of therapy; there were no responders among patients who did not respond in the first two courses and continued to receive a third course of IL-2 (Lindsey et al. 2000). Therefore, as most tumor shrinkage is seen after one course of IL-2, and treatment tolerance and number of doses administered decrease with successive courses of IL-2 (Marroquin et al. 2000), patients with stable disease (either non-responders or following an initial response) rarely benefit from receiving more than 2 courses of therapy.

The side effects associated with IL-2 administration affect nearly every organ system and can range from mild to potentially life threatening symptoms. The duration of each cycle of treatment is determined by each patient's tolerance of side effects, which may vary in intensity and time of onset with each successive cycle. As the side effects are generally reversible by holding treatment, emphasis is placed on determining the safety of proceeding with therapy. This mandates that careful assessments be performed prior to each dose of IL-2. Doses may be withheld to allow for recovery of side effects, many of which follow a cyclical pattern following the dose; however, dosage reductions are not performed. Withheld doses are not replaced and despite dose interruptions, treatment does not extend beyond the scheduled 5-day (maximum 14 doses) treatment cycle. A delay of 24 h or more between doses is generally an indication to discontinue that particular cycle of treatment.

Practical guidelines for the safe administration of high-dose IL-2 have been published (Schwartzentruber 2001). The process begins with careful patient selection. Patients should not have major cardiac, pulmonary, renal, hepatic or medical illnesses and should have a good performance status (ECOG 0 or 1). It is important to screen for occult cardiac disease and for this reason, everyone 50 years of age or older should undergo cardiac stress testing. Evidence of ischemic heart disease is a contraindication to high-dose IL-2. Generally patients with active brain metastases or impending spinal cord compression are not good candidates for high-dose IL-2. Published clinical pathways offer practical help in the assessment of patients before, during and after IL-2 administration (Mavroukakis et al. 2001). Due to the complexity of treatment, high-dose IL-2 is generally administered by doctors and nurses that have received special training in its administration and are familiar with its use.

Following high-dose intravenous bolus injection, the first manifestations of IL-2 side effects can begin with fever and chills within 2–4 h of receiving the first dose (Schwartzentruber 2000). The scheduled administration of antipyretics is necessary to control these side effects. Moderate hypotension and tachycardia develop 2–3 h after the first dose of IL-2 and will persist for the remainder of the treatment. As the end of the cycle nears, additional fluids and vasopressors may be needed to maintain hemodynamic stability. Oliguria frequently develops in the first 24 h and requires additional fluids to restore urine output to a minimum safe level of 20 cc per hour or about 160 cc per 8 h period. The use of fluids to maintain renal perfusion and minimize hypotension, contribute to peripheral edema, pulmonary congestion and weight gain. Patients may gain 10–15 pounds of water weight during treatment and the use of diuretics after IL-2 dosing is stopped is important to initiate the return to baseline weight. When treating hypotension and

oliguria with fluids, it is important to limit fluid boluses to 1.5 l of crystalloid per day in addition to maintenance requirements because fluids compound the manifestation of the capillary leak syndrome. Nausea, vomiting and diarrhea are frequent gastrointestinal side effects and become most pronounced near the end of the treatment cycle. These can typically be controlled with aggressive use of anti-emetics and anti-diarrheals. Dermatitis with erythema, desquamation and subsequent pruritis is frequent and requires diligent application of topical moisturizers and frequently oral antipyretics. Neurologic side effects such as confusion, disorientation, agitation or restlessness occur in up to 20% of patients. This side effect can progress even in the absence of additional treatment; therefore, this represents an indication for withholding therapy in order to prevent the onset of neurologic consequences that can delay treatment recovery and discharge. Progressive transient elevations in creatinine, hyperbilirubinemia and thrombocytopenia are frequent laboratory abnormalities observed, but largely do not require intervention. Cardiac arrhythmias including atrial flutter or fibrillation can occur near the end of treatment, and are usually an indication to stop treatment for that cycle. Myocarditis presenting with elevations of CPK MB or troponin is seen in up to 5% of patients, occurs at the end of the cycle of treatment (frequently during the recovery phase) and is generally asymptomatic. While this toxicity does not preclude subsequent cycles of IL-2, care should be taken that the enzyme elevations return to normal and there is no associated cardiac dysfunction prior to discharge from the hospital. Prior to subsequent administration of IL-2, cardiac evaluation must demonstrate complete recovery of function.

There are many interventions and medications that help control the side effects of IL-2 (Schwartzentruber 2001). Though steroids abrogate many of these toxicities, they are not used because of the possibility of interfering with the anti-tumor response (Vetto et al. 1987; Mier et al. 1990). When a cycle of treatment is stopped, most side effects rapidly reverse and patients can be discharged from the hospital 1–3 days after discontinuation of IL-2.

Clinical Efficacy of High-Dose IL-2 Alone

Currently high-dose IL-2 given by intravenous bolus injection is an FDA approved regimen for treatment of patients with metastatic melanoma. High-dose IL-2 has resulted in an overall clinical response rate of 15–16% in patients with metastatic melanoma (Table 1). The combined experience in the US has demonstrated a complete response rate of 6.3% in 270 patients and a partial response rate of 9.6% (Atkins et al. 2000). The median duration of all responses was 8.9 months, with the median not yet reached for complete responses (range of 2.5 to greater than 122 months) and the median for partial responses lasting 5.9 months (range of 1.5 to greater than 111 months) (Fig. 1). In this report, the median duration of complete response was at least 59 months, and 59% of complete responders remained in complete response. Of note, no patients exhibiting response lasting longer than 30 months experienced a relapse, suggesting that such patients may exhibit long term freedom from melanoma recurrence (Figs. 2, 3, and 4).

Table 1 Clinical responses with high-dose (600,000–720,000 IU/kg/dose) intravenous bolus IL-2 alone every 8 h

Author (Reference)	Evaluable patients	Response			Median duration of response (months)
		CR (%)	PR (%)	Total (%)	
Parkinson et al. (1990)	46	2 (4.3)	8 (17.4)	10 (21.7)	8
Rosenberg et al. (1998b)	182	12 (6.6)	15 (8.2)	27 (14.8)	16
Atkins et al. (2000)[a]	270	17 (6.3)	26 (9.6)	43 (15.9)	8.9

CR complete response, *PR* partial response

[a]Includes some patients from references (Parkinson et al. 1990; Rosenberg et al. 1998b).

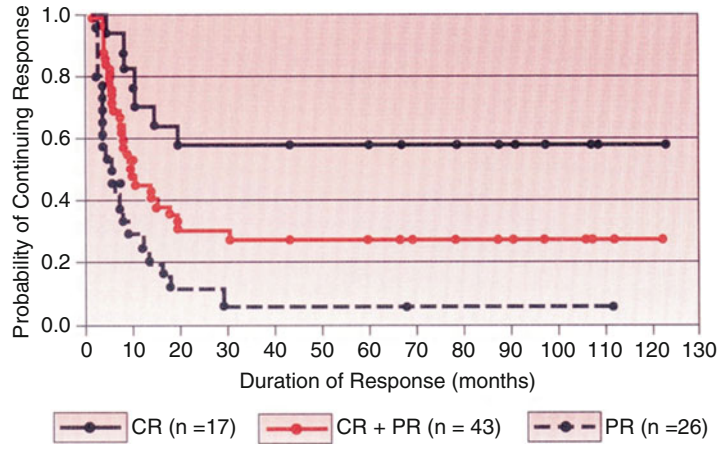

Fig. 1 Kaplan-Meier estimate of response durations for patients with metatatic melanoma achieving complete and partial responses or both (Atkins et al. 2000)

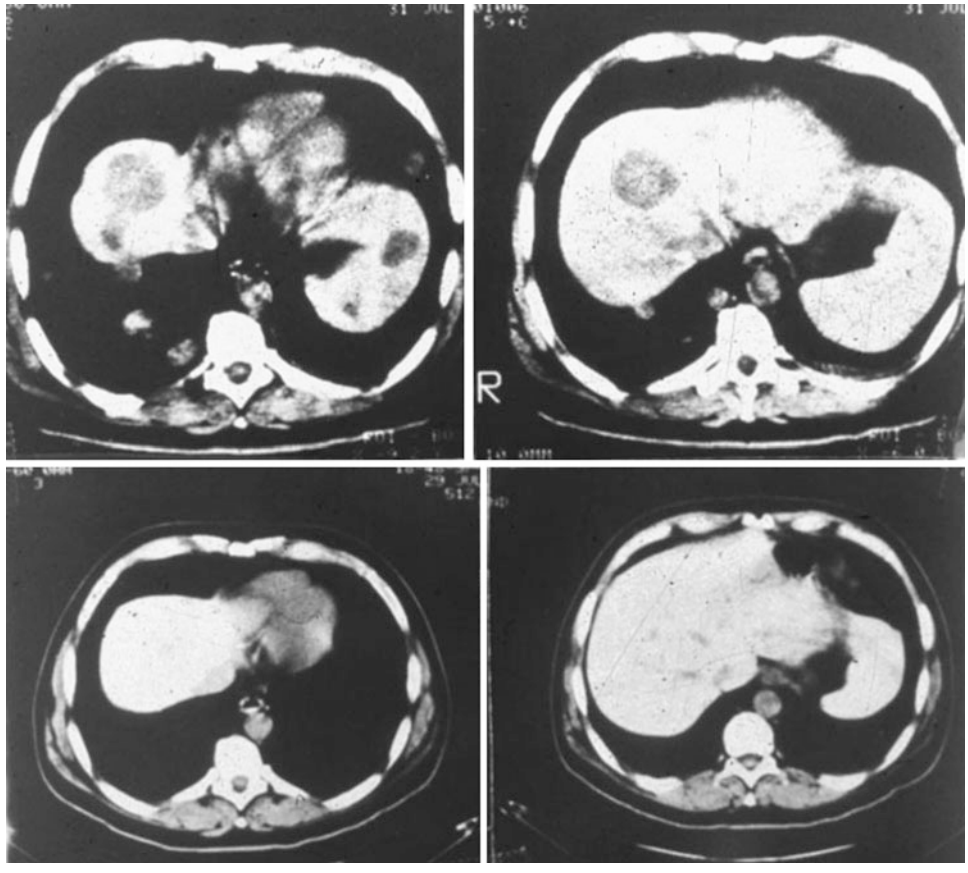

Fig. 2 Patient with metastatic melanoma experiencing complete regression of liver and spleen metastases following high-dose IL-2 (initially intraperitoneal and subsequently intravenous). Top scans are before therapy and bottom scans are 2 years later

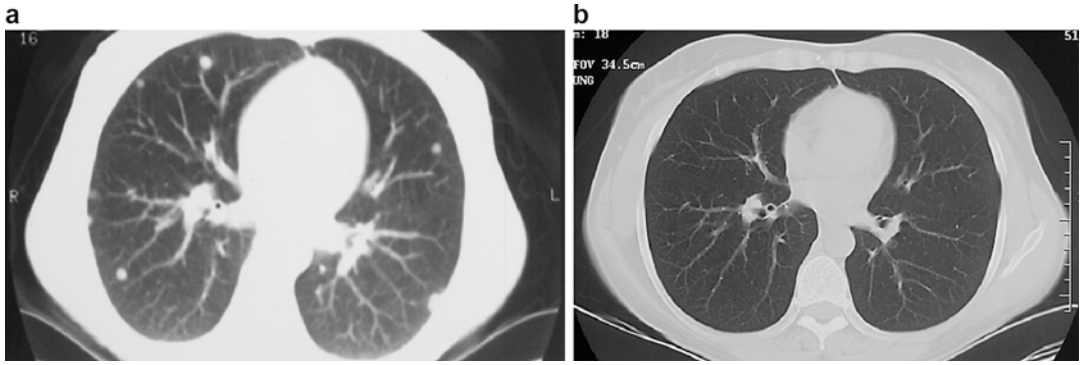

Fig. 3 Patient with metastatic melanoma experiencing complete regression of lung metastases following high-dose IL-2. (**a**) before therapy; (**b**) 12 years later

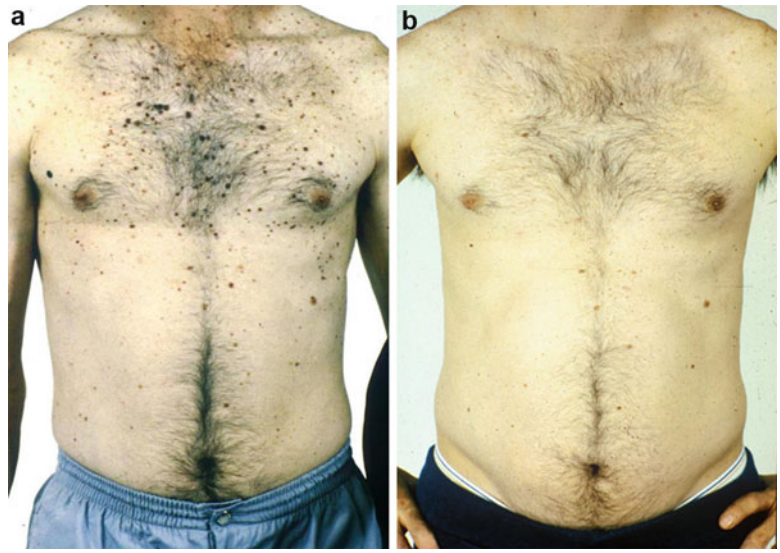

Fig. 4 Patient with metastatic melanoma experiencing complete regression of cutaneous metastases following high-dose IL-2 (with LAK cells). (**a**) before therapy; (**b**) after therapy

The largest single institution experience in the US, the National Cancer Institute, observed very similar response rates in 182 patients (Rosenberg et al. 1998b) to those reported in the above combined series of 270 (80% of the NCI patients were included in the combined report). Ten of 12 patients achieving a complete response at the NCI, remained in complete response from 12 to greater than 148 months. Complete responses have been observed at all sites, including visceral and cutaneous/subcutaneous sites. Previous treatment with low-dose IL-2 or biochemotherapy has not precluded response to high-dose IL-2 as demonstrated in 2 series with 15–19% response rates after failure of these prior therapies (Weinreich and Rosenberg 2002; Tarhini et al. 2007). On the other hand, the response rate in patients who received prior interferon alpha therapy was somewhat less than in patients without such prior treatment (13% vs. 21%, $p = 0.084$) (Weinreich and Rosenberg 2002).

Patients who relapse after achieving a response to high-dose IL-2, are generally not retreated because they rarely respond again. In a series of 33 patients with melanoma who relapsed after responding to various IL-2-based regimens, only 1 responded to the same treatment (Lee et al. 1998). Specifically, none of the 8 patients that initially responded to high-dose IL-2 alone re-responded to that treatment. However,

4 patients re-responded to IL-2 when given with tumor infiltrating lymphocytes (Lee et al. 1998).

A more contemporary series was published several years ago and reported the outcomes of 208 patients treated with high-dose IL-2 at one of two centers, MD Anderson Cancer Center (MDACC) and Beth Israel Deaconess Medical Center (BIDMC), between 2003 and 2009 (Joseph et al. 2012a). The vast majority, 88%, had Stage IV (as opposed to unresectable Stage III) disease as well as a normal LDH (79%). The response rate was similar to the historical datasets, with complete responses and partial responses occurring in 6% and 13% of patients, respectively. Additionally, with a median follow up of 2.5 years in surviving patients, the median progression free survival was 104 days and median overall survival 22.8 months. Further, the estimated 5-year survival was 39%. A second, contemporary multicenter study, enrolled 170 patients treated with standard of care high-dose IL-2 from 2009 to 2014 at 15 centers within the Cytokine Working Group (Sullivan et al. 2016). The complete and partial response rates of 5.4% and 13.3%, respectively, were similar to other historical and contemporary cohorts. The median progression free and overall survival were 2.5 and 21.3 months and, more importantly, the 2-year rate of being progression free was 12.4%. Finally, a multicenter series, also reporting on 170 patients with metastatic melanoma from the PROCLAIM database, reported complete and partial response rates of 5% and 10%, respectively, with a median overall survival of 19.6 months with over 43 months median follow up (Alva et al. 2016). Of note, the overall survival data with all three of these datasets, which are non-overlapping, are remarkably similar and far better than historical data, likely reflecting the general improvements in therapy for these patients over the past decade.

Clinical Efficacy of Alternate Doses, Routes and Schedules of IL-2 Alone

In order to avoid the toxicity of high-dose IL-2, lower doses of IL-2 given by a variety of routes, doses and schedules have been explored in many phase I/II studies (Table 2). The different designs of these studies and the small numbers of patients they include make it difficult to draw meaningful conclusions about clinical efficacy. Bolus IL-2 given 3 days per week (as opposed to every 8 h) (Whitehead et al. 1991; Quan and Quan 2003) or manufactured by Hoffman La-Roche (Sparano et al. 1993) or given in low doses (Marolda et al. 1987) has not appeared to be as effective as high-dose bolus IL-2. No complete responses were seen in these studies. The initial use of PEG-IL-2 (polyethylene glycol modified IL-2) to prolong the circulating half-life of IL-2 did not show superior activity to high-dose IL-2 alone in a randomized trial, resulting in a response rate of 11% in 35 treated patients (Yang et al. 1995).

The highest doses of IL-2 given by continuous intravenous infusion (12–18 MIU/m^2/day) in 5 small series of patients (15–31 patients each) have resulted in clinical response rates of 6–33% (Perez et al. 1991; Dillman et al. 1997; Legha et al. 1996; Hidalgo et al. 1996; Dorval et al. 1992). The collective experience with high-dose IL-2 alone administered primarily by continuous infusion in Europe has demonstrated an overall response rate of 15% in 117 patients (Keilholz and Eggermont 2000). The median survival in this study was 7.5 months, which appears lower than the 12 month median survival reported for patients treated with high-dose bolus IL-2 in the initial U.S. experience (Atkins et al. 2000). Lower doses of continuous infusion IL-2 or prolonged duration of infusion (up to 90 days) have not consistently resulted in higher response rates (Richards et al. 1988; Creekmore et al. 1989; Engelhardt et al. 1997; Quan et al. 2005; Goldstein et al. 1989; Oliver et al. 1989; Vlasveld et al. 1992, 1994; Caligiuri et al. 1991).

In contrast to its frequent use in patients with renal cell carcinoma, subcutaneous IL-2 has been infrequently used to treat patients with metastatic melanoma and there is very little evidence of clinical activity (Atzpodien et al. 1990a; Stein et al. 1991; Angevin et al. 1995; Tagliaferri et al. 1998; Ahmed et al. 1996; Leahy et al. 1992). The largest study with subcutaneous administration of IL-2 in 133 patients noted an overall response rate of 4% and concluded that survival was inferior to a similar regimen combined with histamine (Agarwala et al. 2002). There is also very little experience with intramuscular and intralymphatic

Table 2 Clinical efficacy of alternate routes, doses and schedules of IL-2 alone

Author (Reference)	Evaluable patients	Regimen	Response		
			CR	PR	Total (%)
Whitehead et al. (1991)	42	B 3 day/week (high, Chiron)	0	4	4 (10)
Quan and Quan (2003)	7	B 3 day/week (intermediate)	0	2	2 (29)
Sparano et al. (1993)	44	B (high, Hoffman)	0	2	2 (5)
Marolda et al. (1987)	12	B (low, Biogen)	0	0	0
Yang et al. (1995)	35	B/PEG-IL-2 (high,Chiron)	2	2	4 (11)
Thompson et al. (1987)	≤5	CIV 1 day/week	0	0	0
Richards et al. (1988)	4	CIV 1 day/week	0	0	0
Creekmore et al. (1989)	12	CIV 1 day/week	0	2	2 (17)
Perez et al. (1991)	17	CIV 1 day/week	0	1	1 (6)
Engelhardt et al. (1997)	2	CIV 2 day/week	0	0	0
Dillman et al. (1997)	18	B × 1, CIV 3 day/week	2	2	4 (22)
Quan et al. (2005)	10	CIV 3 day/week +/−B × 1	1	5	6 (60)
Sosman et al. (1988)	≤7	CIV 4 day/week +/−B	0	0	0
Punt et al. (1992)	17	CIV 4 day/week	0	0	0
Legha et al. (1996)	31	CIV 4 day/week	1	6	7 (22)
Sondel et al. (1988)	5	CIV 4 day/week	0	0	0
Goldstein et al. (1989)	4	CIV 4 day/week	0	0	0
Hidalgo et al. (1996)	15	CIV 5 day/week	0	2	2 (13)
Dorval et al. (1992)	24	CIV 5 day/week	0	8	8 (33)
Oliver et al. (1989)	7	CIV 5 day/week	0	0	0
Keilholz and Eggermont (2000)	117	CIV 1–5 day/week, IV	NM	NM	17 (15)
Lotze et al. (1986b)	2	CIV × 7–21 day	0	0	0
Vlasveld et al. (1992)	13	CIV × ≥21 day	0	1	1 (8)
Vlasveld et al. (1994)	15	CIV × 21 day	0	0	0
Caligiuri et al. (1991)	2	CIV × 90 day	NM	NM	NM
Atzpodien et al. (1990a)	2	SQ	0	0	0
Stein et al. (1991)	6	SQ	0	0	0
Angevin et al. (1995)	2	SQ	0	0	0
Tagliaferri et al. (1998)	6	SQ	0	0	0
Ahmed et al. (1996)	<17	SQ	0	2	2 (>12)
Agarwala et al. (2002)	133	SQ	2	3	5 (4)
Leahy et al. (1992)	5	SQ continuous	0	0	0
Urba et al. (1990)	4	IM	0	0	0
Galvani et al. (1992)	4	Intralymphatic	0	0	0
Sarna et al. (1994)	7	Intralymphatic	0	0	0
Lotze et al. (1986a)	3	Intraperitoneal	0	1	1 (33)
Thatcher et al. (1989)	31	Intraarterial, IV	0	4	4 (13)
Klasa et al. (1990)	6	Intraarterial	0	0	0
Heimans et al. (1991)	1	Intracranial	0	0	0
List et al. (1992)	9	Intracranial	NM	NM	NM
Rosener et al. (1993)	1	Intracranial	NM	NM	NM
Samlowski et al. (1993)	1	Intracranial	0	0	0
Lotze et al. (1986b)	2	Intralesional	0	0	0
Radny et al. (2003)	24	Intralesional	0	0	0[a]

(*continued*)

Table 2 (continued)

Author (Reference)	Evaluable patients	Regimen	Response		
			CR	PR	Total (%)
Green et al. (2007)	10	Intralesional	0	0	0[a]
(Gutwald et al. 1994)	2	Peritumoral	NM	NM	NM
Enk et al. (2000)	27	Inhalational[b]	5[a]	8[a]	13 (48)[c]
Bernatchez et al. (2017)	7	SQ (NKTR-214)	0	0	0

CR complete response, *PR* partial response, *SQ* subcutaneous, *CIV* continuous intravenous, *IV* intravenous, *B* bolus, *IM* intramuscular, *PEG* polyethylene glycol modified, *NM* not mentioned
[a]Response in injected lesions only; no systemic responses
[b]Concurrent dacarbazine given
[c]Lung response only; no extrapulmonary responses

administration of IL-2 and there have been no reported clinical responses when given by these routes (Urba et al. 1990; Sarna et al. 1994; Galvani et al. 1992). Intraperitoneal IL-2 (Lotze et al. 1986a) was evaluated in the early studies of IL-2 and was not continued because of the technical challenges and side effects associated with that route of administration. The novel approach of delivering IL-2 into the splenic artery (Klasa et al. 1990; Thatcher et al. 1989) resulted in clinical responses only when combined with high doses of bolus IL-2 intravenously (4 of 31 patients, 13%), but yielded no complete responses (Thatcher et al. 1989). Intracranial administration of IL-2 has no reported clinical responses (Heimans et al. 1991; List et al. 1992; Rosener et al. 1993; Samlowski et al. 1993). The direct injection of IL-2 into a tumor metastasis or the surrounding tissue has resulted in complete or partial regression of 50–90% of the injected lesions without evidence of systemic response (Lotze et al. 1986b; Radny et al. 2003; Green et al. 2007; Gutwald et al. 1994). Delivery of IL-2 directly to tumors in the lung by the inhalational route (with concurrent dacarbazine) has resulted in regression of pulmonary lesions in 13 of 27 (48%) patients but no responses in metastatic sites outside the lung (Enk et al. 2000).

One of the most striking observations that emerges from the reports of IL-2 alone when used in regimens other than by high-dose bolus, is the paucity of reports of complete and durable responses. Randomized trials to support this observation have not been performed in patients with melanoma; however, such a trial comparing high-dose IL-2 to lower doses of IL-2 in patients with metastatic kidney cancer has been reported (Yang et al. 2003). This trial clearly demonstrated lower toxicity of these alternate regimens but also lower clinical response rates and in particular, fewer complete and/or durable responses.

With the development of newer immunotherapies that target immune checkpoints such as cytotoxic T-lymphocyte associated antigen 4 (CTLA-4), programmed death 1 receptor (PD-1) and its ligand (PD-L1) (See chapter ▶ "Checkpoint Inhibitors in the Treatment of Metastatic Melanoma"), there has been renewed interest in other active immunotherapy approaches such as cytokines. One of these has been the exploration of IL-2 modifications to favor T-effector/NK cell activity without T-regulatory activation. The most developed of these approaches has been a novel pegylation technique that culminates in a molecule of 6 polyethylene glycol (PEG) moieties and IL-2. After introduction into the patient, the PEGs are irreversibly cleaved one by one and lead to the conversion of the 6-PEG-IL-2 prodrug, known as NKTR-214, into a biologically active 2-PEG-IL-2 or 1-PEG-IL-2 molecule that purportedly preferentially binds to T-effector and/or NK cell IL-2 receptors as opposed to T regulatory cell IL-2 receptors (Charych et al. 2016, 2017). In the tumor microenvironment, this leads to an influx

of effector cells, as well as a compensatory expression by the tumor of PD-L1. NKTR-214 as a single-agent was not associated with responses in any of the 28 patients, including 7 with melanoma, in the dose-escalation phase I study monotherapy trial (Bernatchez et al. 2017). However, in serial biopsies, there was evidence of an influx of PD-1, CD8 dual positive T cells, setting the stage for potential combination therapy with anti-PD-1/PD-L1 monoclonal antibodies (described below).

Clinical Efficacy of IL-2 Combined with Interferon Alpha

A variety of routes, doses and schedules combining IL-2 and interferon alpha have been evaluated in primarily phase I/II studies (Table 3). Only one randomized trial has been performed and it compared the efficacy of IL-2 plus interferon alpha to IL-2 alone (Sparano et al. 1993). In this study, high-dose IL-2 (Hoffman La-Roche, Nutley, NJ) response rates (5%) were no different than bolus

Table 3 Clinical responses with combinations of IL-2 and IFN α

Author (Reference)	Evaluable patients	Regimen		Response		
		IL-2	IFN α	CR	PR	Total (%)
Marincola et al. (1995b)	82	B (escalating)	B	6	14	20 (24)
Kruit et al. (1996)	17	B (mod-high)	B (5 day)	2	5	7 (41)
Kruit et al. (1996)	25	B (mod-high)	B (3 day)	0	5	5 (20)
Sparano et al. (1993)	41	B (high)	B	0	4	4 (10)
Sznol et al. (1990)	7	B (escalating)	B	0	0	0
Karp (1998)	38	B (low)	SQ	1	5	6 (16)
Budd et al. (1992)	17	B 3 day/week	IM	2	3	5 (29)
Huberman et al. (1991)	9	IV 2 h	IM	0	1	1 (11)
Keilholz et al. (1993)	27	CIV	SQ	1	4	5 (18)
Oldham et al. (1992)	66	CIV	SQ	NM	NM	7 (11)
Kruit et al. (1995)	51	CIV	SQ	1	7	8 (16)
Pichert et al. (1991)	8	CIV	SQ	0	2	2 (25)
Thomas et al. (1992)	8	CIV	SQ	0	0	0
Veelken et al. (1992)	8	CIV	SQ	0	2	2 (25)
Hellstrand et al. (1994)	7	CIV	SQ	0	1	1 (14)
Maxwell et al. (1993)	6	CIV	SQ	0	1	1 (17)
Whitehead et al. (1993)	14	CIV	SQ/IM	0	0	0
Eton et al. (1996)	23	CIV	IM	0	2	2 (8)
Mittelman et al. (1990)	14	CIV	IM	0	0	0
Bukowski et al. (1990)	3	CIV	IM	0	0	0
Lee et al. (1989)	10	CIV	IM	1	3	4 (40)
Bergmann et al. (1990)	11	CIV (post IFN)	SQ	0	3	3 (27)
Keilholz et al. (1993)	27	Decrescendo	SQ	3	8	11 (41)
Keilholz et al. (1997)	66	Decrescendo	SQ	4	8	12 (18)
Eton et al. (2000)	21	Decrescendo	SQ	0	0	0
Atzpodien et al. (1990b)	7	SQ	SQ	0	1	1 (14)
Vuoristo et al. (1994)	4	SQ	SQ	0	0	0
Gause et al. (1996)	12	SQ	SQ	0	0	0
Hidalgo et al. (1996)	11	SQ	SQ	0	2	2 (18)
Rosso et al. (1992)	7	SQ	IM	0	1	1 (14)
De Braud et al. (1993)	15	SQ	IM	0	0	0
Castello et al. (1993)	15	SQ	IM	0	0	0

IFN α interferon alpha, *CR* complete response, *PR* partial response, *SQ* subcutaneous, *CIV* continuous intravenous, *B* bolus, *IM* intramuscular, *NM* not mentioned

IL-2 at a slightly lower dose combined with interferon alpha (10% response rate). The largest single study evaluating escalating doses of bolus IL-2 (Hoffman La-Roche) and interferon alpha in 82 patients resulted in overall response rates of 24% (Marincola et al. 1995b). It appeared that higher doses of each cytokine resulted in higher response rates but the higher toxicity at those doses made them impractical. Similar observations were made in a separate study in which the response rate in 17 patients was 41% but the cardiac and neurologic toxicity of this regimen was excessive (Kruit et al. 1996). A less intense regimen by the same investigators reduced these toxicities but also resulted in a drop in response rate in 25 patients to 20% (Kruit et al. 1996). Bolus IL-2 given three times weekly or at lower doses combined with intramuscular or subcutaneous interferon resulted in clinical response rates of 29% and 16% respectively (Budd et al. 1992; Karp 1998) and were not clearly superior to IL-2 alone. Escalating bolus doses or 2 h intravenous infusions of IL-2 have not shown enhanced activity (Sznol et al. 1990; Huberman et al. 1991).

Many studies have evaluated continuous intravenous infusion IL-2 along with interferon administered subcutaneously or intramuscularly. The three largest studies with continuous infusion IL-2 involved 27, 66 and 51 patients, and demonstrated response rates of 18%, 11% and 16% respectively (Keilholz et al. 1993; Oldham et al. 1992; Kruit et al. 1995). Investigators in Europe piloted a regimen of decrescendo IL-2 in order to reduce toxicity and observed a response rate of 41% in 27 patients (Keilholz et al. 1993). The experience with this regimen of interferon alpha and decrescendo IL-2 was not confirmed in a larger EORTC series of 66 patients exhibiting a response rate of 18% (Keilholz et al. 1997) or in 21 patients in a US study in which no responses were seen (Eton et al. 2000). Other smaller series of continuous intravenous IL-2 with interferon have not demonstrated superiority (Pichert et al. 1991; Thomas et al. 1992; Veelken et al. 1992; Maxwell et al. 1993; Whitehead et al. 1993; Eton et al. 1996; Mittelman et al. 1990; Bukowski et al. 1990; Lee et al. 1989; Bergmann et al. 1990) to reported results with the high dose bolus IL-2 regimen. Outpatient regimens of subcutaneous IL-2 and subcutaneous or intramuscular interferon are generally the least toxic and have yielded response rates of 0–18% (Hidalgo et al. 1996; Atzpodien et al. 1990b; Vuoristo et al. 1994; Gause et al. 1996; Rosso et al. 1992; De Braud et al. 1993; Castello et al. 1993).

The lessons learned from all of these studies were that combining IL-2 and interferon alpha generally resulted in additive toxicities preventing their use at full doses due to unacceptable toxicity. When lower doses of each cytokine were used to reduce toxicity, clinical response rates were not clearly improved over those of high-dose IL-2 alone. In particular, the complete responses characteristic of high-dose bolus IL-2 administration were less frequently observed. Hence, with the added toxicities of the combined regimens and no clear response advantage, the combination of IL-2 and interferon alpha has not been widely used outside of the context of biochemotherapy regimens) that combined IL-2 +/− interferon alpha with dacarbazine and/or cisplatin-based cytotoxic chemotherapy. While these approaches showed higher response rates and median PFS compared to chemotherapy alone in patients with stage IV melanoma, they largely failed to improve median OS (Atkins et al. 2008) and, therefore, have largely been abandoned.

Clinical Efficacy of IL-2 Combined with Other Cytokines, Immune Modulators, Antibodies or Vaccines

When IL-2 has been combined with various cytokines such as GM-CSF (Smith et al. 2003; Ridolfi et al. 2001), interferon β (Krigel et al. 1988), interferon γ (Weiner et al. 1991; Viens et al. 1992; Kim et al. 1996; Margolin et al. 1992; Taylor et al. 1992), IL-1 β (Triozzi et al. 1995), IL-4 (Olencki et al. 1996), IL-12 (Gollob et al. 2003), or TNF α (Rosenberg et al. 1989; Negrier et al. 1992; Krigel et al. 1995; Dillman et al. 1993) no obvious increase in clinical response rates have been observed above that observed historically with IL-2 alone (Table 4). Similarly, the combination of IL-2 and various immune modulators such

Table 4 Clinical responses with combinations of IL-2 and cytokines

Author (Reference)	Evaluable patients	Regimen		Response		
		IL-2	Cytokine	CR	PR	Total (%)
GM-CSF						
Smith et al. (2003)	8	B (low)	SQ	0	0	0
Ridolfi et al. (2001)	16	SQ	IL	0	2	2 (13)
Interferon β						
Krigel et al. (1988)	3	SQ/IV	IV	0	0	0
Interferon γ						
Weiner et al. (1991)	7	B (3 × week)	SQ	0	1	1 (14)
Viens et al. (1992)	11	B	SQ	1	1	2 (18)
Kim et al. (1996)	13	B (high)	SQ	1	1	2 (15)
Margolin et al. (1992)	6	B	IM	0	1	1 (17)
Taylor et al. (1992)	7	B	IM	0	1	1 (14)
IL-1 β						
Triozzi et al. (1995)	3	CIV	B	0	1	1 (33)
IL-4						
Olencki et al. (1996)	13	B	SQ	0	2	2 (15)
IL-12						
Gollob et al. (2003)	9	SQ	IV	0	1	1 (11)
TNF α						
Rosenberg et al. (1989)	15	B	B	1	0	1 (7)
Negrier et al. (1992)	5	CIV	B	0	0	0
Krigel et al. (1995)	7	CIV	B	1	1	2 (29)
Dillman et al. (1993)	13	CIV	CIV	NM	NM	2 (15)

CR complete response, *PR* partial response, *SQ* subcutaneous, *IL* intralesional, *CIV* continuous intravenous, *B* bolus, *IV* intravenous, *IM* intramuscular, *NM* not mentioned

as cyclophosphamide (Rosenberg et al. 1989; Mitchell et al. 1988; Lindemann et al. 1989; Verdi et al. 1992; Oldham et al. 1991), famotidine (Quan et al. 2006), flavone acetic acid (Thatcher et al. 1990; O'reilly et al. 1993; Holmund et al. 1995), histamine (Agarwala et al. 2002; Hellstrand et al. 1994; Lindner et al. 2004; Schmidt et al. 2002; Middleton et al. 2007), levamisole (Ahmed et al. 1996; Creagan et al. 1997), melatonin (Lissoni et al. 1994, 1997), radiation (Lange et al. 1992; Safwat et al. 2005), SRL 172 (*Mycobacterium vaccae*) (Nicholson et al. 2003) or taurolidine (O'brien et al. 2006) have not improved the clinical activity of IL-2 (Table 5). Monoclonal antibodies such as 9.2.27 (Rosenberg et al. 1989), ch14.18 (Albertini et al. 1997), 14.G2a (Albertini et al. 1996), R24 (Bajorin et al. 1990; Soiffer et al. 1997; Nasi et al. 1997; Alpaugh et al. 1998), ch14.14 plus R24 (Choi et al. 2006) or anti-CD3 (Sosman et al. 1993; Hank et al. 1995; Sosman et al. 1995; Buter et al. 1993) when combined with IL-2 have failed to significantly augment the response rate of IL-2 as well (Table 6).

An apparent increase in clinical activity was noted when high-dose IL-2 was combined with gp100:209–217(210 M) peptide vaccine in a phase II trial of 31 patients, resulting in a 42% response rate (Rosenberg et al. 1998a). Three phase II studies using slightly different schedules of high dose IL-2 combined with this vaccine in 39–42 patients each produced response rates of 13–24% (Sosman et al. 2008). This strategy was subsequently studied in a randomized multi-institutional phase III trial comparing vaccine plus IL-2 to high-dose IL-2 alone. The results of this trial showed a near tripling of response rate (16% versus 6%) in patients treated with vaccine plus high-dose IL-2, but the low rate of response in the control arm limits the impact and applicability of these findings (Schwartzentruber et al. 2011).

Table 5 Clinical responses with combinations of IL-2 and immune modulators

Author (Reference)	Evaluable patients	Regimen		Response		
		IL-2	Other	CR	PR	Total (%)
Cyclophosphamide						
Rosenberg et al. (1989)	13	B (high)	IV	0	2	2 (15)
Mitchell et al. (1988)	24	IV	IV	1	5	6 (25)
Lindemann et al. (1989)	18	IV	IV	0	2	2 (11)
Verdi et al. (1992)	23	CIV	IV	0	1	1 (4)
Oldham et al. (1991)	8	CIV	IV	0	0	0
Famotidine						
Quan et al. (2006)	12	CIV	IV	0	3	3 (25)
Flavone acetic acid						
Thatcher et al. (1990)	34	IA, IV	IV	1	4	5 (15)
O'reilly et al. (1993)	21	CIV	IV	1	2	3 (14)
Holmund et al. (1995)	8	CIV	IV	0	0	0
Histamine						
Hellstrand et al. (1994)	8	CIV	SQ + IFN α SQ	0	4	4 (50)
Lindner et al. (2004)	27	SQ or B	SQ + IFN α SQ	1	3	4 (15)
Agarwala et al. (2002)	150	SQ	SQ	1	4	5 (3)
Schmidt et al. (2002)	41	SQ	SQ	0	2	2 (5)
Middleton et al. (2007)	119	SQ	SQ + IFN α SQ	3	12	15 (13)
Levamisole						
Ahmed et al. (1996)	<17	SQ	PO	0	0	0
Creagan et al. (1997)	19	SQ	PO	0	0	0
Melatonin						
Lissoni et al. (1994)	1	SQ	PO	0	0	0
Melatonin and methoxytryptophol						
Lissoni et al. (1997)	1	SQ	PO	0	0	0
Radiation						
Lange et al. (1992)	10	B (high)	Regional	0	1	1 (10)
Safwat et al. (2005)	45	SQ	LTBI	0	2	2 (4)
SRL 172 (Mycobacterium vaccae)						
Nicholson et al. (2003)	16	SQ	ID	0	3	3 (19)
Taurolidine						
O'brien et al. (2006)	11	CIV	CIV	2	0	2 (18)

CR complete response, *PR* partial response, *SQ* subcutaneous, *ID* intradermal, *CIV* continuous intravenous, *B* bolus, *IV* intravenous, *PO* oral, *IA* intra-arterial, *LTBI* low-dose total body irradiation

As described above, inhibitors of immune checkpoints have demonstrated activity in and become FDA-approved for the treatment of patients with advanced melanoma (Hodi et al. 2010). The first of these developed was the anti-CTLA-4 monoclonal antibody ipilimumab, however the anti-PD1 antibodies pembrolizumab and nivolumab followed soon after (Robert et al. 2015a, b). While combination studies have been or are being performed, there is data on the sequencing of immune checkpoint inhibitors and high-dose IL-2 that helps to determine the potential for sequencing of IL-2 with other therapies. The first report involved the safety of interleukin-2 following ipilimumab come out of the National Cancer Institute (NCI) (Smith et al. 2007). Of 22 patients treated with IL-2 following ipilimumab, three developed bowel perforation; this in contrast to the four perforations in 198 patients treated at the NCI with ipilimumab from 2002 to 2005 and eight out of 1797 patients treated with high-dose IL-2 there from 1998 to

Table 6 Clinical responses with combinations of IL-2 and monoclonal antibodies

Author (Reference)	Evaluable patients	Regimen		Response		
		IL-2	Antibody	CR	PR	Total (%)
Rosenberg et al. (1989)	12	B (high)	9.2.27, IV	0	0	0
Albertini et al. (1997)	24	CIV	ch14.18, IV	1	1	2 (8)
Albertini et al. (1996)	4	CIV	14.G2a, IV	NM	NM	NM
Bajorin et al. (1990)	20	IV	R24, IV	0	1	1 (5)
Soiffer et al. (1997)	18	CIV	R24, IV	0	0	0
Nasi et al. (1997)	18	SQ	R24, CIV	0	0	0
Alpaugh et al. (1998)	16	SQ + IFN SQ	R24, CIV	0	0	0
Choi et al. (2006)	18	CIV	ch14.18, R24, IV	0	2	2 (11)
Sosman et al. (1993)	16	B (high)	Anti-CD3, IV	1	0	1 (6)
Hank et al. (1995)	15	CIV	Anti-CD3, IV	0	0	0
Sosman et al. (1995)	9	CIV	Anti-CD3, IV	0	0	0
Buter et al. (1993)	1	SQ	Anti-CD3, IV	0	0	0
Maker et al. (2005)	36	B (high)	Anti-CTLA-4	3	5	8 (22)

CR complete response, *PR* partial response, *CIV* continuous intravenous, *B* bolus, *IV* intravenous, *SQ* subcutaneous, *NM* not mentioned

2002. A more contemporary series published in 2016, reported on 52 patients treated with high-dose IL-2 after ipilimumab compared to 276 patients treated with IL-2 without prior ipilimumab (Buchbinder et al. 2016). Efficacy was similar, specifically related to overall survival from IL-2 commencement; however two episodes of severe GI-toxicity occurred including one death from bowel perforation in the group receiving high-dose IL-2 following ipilimumab therapy. In another study, the efficacy of ipilimumab after IL-2 was evaluated in a subset (48) of 208 patients treated with high-dose IL-2 from 2003 to 2009 (Joseph et al. 2012a). In these 48 patients subsequently treated with ipilimumab, the clinical benefit of ipilimumab, was similar to contemporary reports of ipilimumab benefit. Specifically, the response rate was approximately 16% with a median progression and overall survival of 2.5 and 12 months, respectively.

The initial study that combined ipilimumab with high-dose IL-2 resulted in a response rate of 22% in 36 patients (Maker et al. 2005), which was seemingly not different than expected with IL-2 alone. However, following the FDA-approval of ipilimumab in 2011, it remained an unsettled question as to whether high-dose IL-2 could be safely given with ipilimumab and whether the combination may be associated with improved outcomes. This study could not be completed due to the emergence of the anti-PD-1 antibodies as superior first line agents in patients with metastatic melanoma. The data from the 46 patients enrolled onto the phase I portion of the ipilimumab plus interleukin-2 study showed that the combination was tolerable and not associated with undue or unexpected toxicity. There remains great excitement in combining high-dose IL-2 with the immune checkpoint inhibitors, specifically anti-PD-1 antibodies, and trials are underway (NCT03476174).

In 2017, the first data from the trial combining NKTR-214 and nivolumab was presented and updated at the 2018 American Society of Clinical Oncology Meeting (Diab et al. 2017, 2018). The initial 11 melanoma patients did remarkably well, with confirmed response in seven and disease control rate, a RECIST-defined response (complete or partial) or stable disease, in ten of 11. With more patients treated (28 reported to be eligible for response), the response rate fell to 50% (14 of 28) and disease control rate to 71% (20 of 28). This trial is ongoing and larger trials based on these preliminary results are being planned (see chapter ▶ "Novel Immunotherapies and Novel Combinations of Immunotherapy for Metastatic Melanoma").

Clinical Efficacy of IL-2 Combined with Targeted Therapy

With the emergence of BRAF-targeted therapy as a useful and life-prolonging strategy for patients with BRAF mutant metastatic melanoma a number of trials were launched to combine BRAF inhibitors with immunotherapy. Preclinical and early clinical studies suggested that BRAF inhibitor therapy was associated with a number of changes in the melanoma tumor microenvironment that might enhance tumor responsiveness to immunotherapy (Boni et al. 2010; Cooper et al. 2013; Frederick et al. 2013). Specifically, antigen presentation increases, immunosuppressive cytokines go down, T cell clonality increases, and an influx of T cells are seen. Building on these findings, a trial of vemurafenib plus high-dose IL-2 was launched in 2012 (NCT01683188) (Mooradian et al. 2018). This trial closed after enrolling six of a planned 43 patients. Two thirds responded, but no long-term durable responses where seen.

Agents to Reduce the Toxicity of IL-2

The safety of high-dose bolus IL-2 has improved as experience with this regimen has grown (Kammula et al. 1998). The diminished intensity of side effects of high-dose IL-2 has also correlated with a decrease in the number of IL-2 doses given in each cycle in some institutions. Despite the reduction of toxicity, this therapy remains a very intensive inpatient regimen. A variety of concomitant medications such as paracetamol, indomethacin and antiemetics are routinely used to control the common side effects of IL-2 (Schwartzentruber 2001). Various other agents have been studied with the hope of ameliorating toxicity. One such example are corticosteroids which have reduced the side effects of IL-2 (Vetto et al. 1987; Mier et al. 1990). However, steroids have abrogated the therapeutic benefit of IL-2 in mice (Papa et al. 1986) and the concomitant use of steroids with IL-2 is strongly discouraged. Patients should be warned not to use steroids either systemically or topically after receiving IL-2 therapy. Agents which have not been clinically beneficial at reducing IL-2 toxicity in patients with melanoma have included: soluble tumor necrosis factor receptor (Trehu et al. 1996; Du Bois et al. 1997), soluble IL-1 receptor (Mcdermott et al. 1998), lisofylline (Margolin et al. 1997), C1 esterase inhibitor (Ogilvie et al. 1994), and CNI-1493 (Atkins et al. 2001). Low dose dopamine induced recovery of impaired renal function in patients receiving continuous infusion IL-2 (Palmieri et al. 1993) but was not significantly beneficial when used prophylactically in a randomized trial of high-dose IL-2 (Cormier et al. 1997). Taurolidine when used in combination with a decrescendo regimen of continuous infusion IL-2 appeared to enhance tolerability in 11 patients (O'brien et al. 2006). *N*-Monomethyl-L-Arginine has demonstrated antihypotensive activity in patients with metastatic renal cell carcinoma receiving IL-2 (Kilbourn et al. 2000) and has been evaluated in patients with melanoma receiving high-dose IL-2, without clear clinical benefit despite having apparent antihypotensive effects (Richard Sherry, personal communication).

Predictors of Clinical Response

The ability to predict which patients will respond to IL-2 therapy is desirable since treatment and toxicity could then be spared of those who are not likely to respond. This is particularly important, given that emergence of more effective therapies to treat advanced melanoma, including BRAF targeted therapy and immune checkpoint inhibitors. Basically, there needs to be compelling evidence to support the use of high-dose IL-2 to forego frontline therapy with the more recently approved agents, and the identification of predictive biomarkers to select patients with a high likelihood of therapeutic response is critical. Traditionally, there have been no in-vitro predictors of response (reviewed in (Schwartzentruber 1993)). ECOG performance status of "0" and not having received prior systemic therapies were factors associated with higher clinical response in 270 patients receiving high-dose IL-2 (Atkins

et al. 1999). Similarly, patients who received no prior immunotherapy were more likely to have a complete response to IL-2 than patients who received prior immunotherapy (Rosenberg et al. 1998b). One report found a significant association of expression of HLA-DQ1 tissue type with clinical response (Rubin et al. 1995), but a larger study of 272 patients found no association between HLA type and response to IL-2 (Marincola et al. 1995a). This latter study did find a correlation between HLA-DR3 and –DR4 alleles and decreased tolerance to IL-2.

Patients with only cutaneous and subcutaneous metastases have a 50% response rate to high-dose IL-2 which is higher than the 15% rate observed in patients with disease at any other site (Chang and Rosenberg 2001; Phan et al. 2001; Royal et al. 1996). The importance of site of disease was confirmed by French investigators; they also found that high levels of C-reactive protein before therapy was associated with a poor response to IL-2 (Tartour et al. 1996). The majority of patients, however, do not have disease confined to the skin or subcutaneous tissue, and sites of disease and measurements of serum markers have not been used to exclude people from therapy.

Though various treatment-related parameters have been associated with higher clinical response, none has been useful in selecting patients for therapy or altering the therapy in progress. For example, responders have received more doses of IL-2 and developed a greater rebound lymphocytosis than non-responders (Rosenberg et al. 1998b; Phan et al. 2001; Royal et al. 1996). Circulating regulatory T cells ($CD4^+$ $CD25^+$) have decreased following administration of high-dose IL-2 but only in those patients achieving a clinical response to therapy (Cesana et al. 2006). Post-treatment biopsy of metastatic lesions has demonstrated greater HLA-DR antigen expression and T cell infiltrate in regressing lesions than in non-responding lesions (Rubin et al. 1989). Autoimmune hypothyroidism (Phan et al. 2001; Krouse et al. 1995) and vitiligo (Phan et al. 2001; Rosenberg and White 1996) have occurred more frequently in responders than non-responders suggesting that tumor response may be similarly mediated by the induction of an autoimmune reaction against the tumor cells. In general, whether these changes are a function of duration of treatment and follow-up rather than a true association with response (i.e. these toxicities take time to evolve and responders receive more courses of treatment and are followed longer than non-responders) may never be settled.

More recently, patients with an elevated LDH were identified as being uniquely unlikely to respond or have long-term benefit from high-dose IL-2, but this appears to be consistent with the general thinking that patients with a better performance status and less aggressive disease will do better whereas those with a poor performance status and/or aggressive disease will do worse.

Research to discover better biomarkers that will predict responsiveness to high-dose IL-2 has been ongoing, and newer technologies are being used to aide in this discovery. For example, the analysis of tumor DNA for activating mutations for BRAF has been standard of care since the approval of vemurafenib, a potent and specific BRAF inhibitor, since 2011 (Flaherty et al. 2010) (See chapter ▶ "Targeted Therapies for BRAF-Mutant Metastatic Melanoma"). However, only 45–50% of patients with cutaneous melanoma have a tumor harboring such a mutation and approximately a quarter have mutations in NRAS, which like BRAF, constitutively activates the mitogen activated protein kinase (MAPK) pathway when mutated (See chapter ▶ "Molecularly Targeted Therapy for Patients with BRAF Wild-Type Melanoma"). Interestingly, the presence or absence of a BRAF mutation is not associated with improved responsiveness to high-dose IL-2; however, the presence of an NRAS mutation was, in an analysis of 103 patients with mutational testing available (Joseph et al. 2012b). This has not been validated in larger studies, but is an intriguing finding. In a smaller analysis of 49 patients, gene expression profiling was performed on archived, pretreatment tumors and a GEP enriched with immune-related genes was associated with a higher response rate than in those with an alternative GEP (Sullivan et al. 2016). Building upon this work, the

IL-2 Select Melanoma trial was launched with a plan to prospectively validate this GEP approach as a predictive marker of benefit in patients with melanoma treated with high-dose IL-2. From 2009 to 2014, 170 patients were enrolled and RNA sequencing was able to be performed on pretreatment, archival blocks from 101 patients. Application of the prior gene expression classification with immune genes was modestly associated with response (normalized enrichment score of −1.70, p value <0.001, false discovery rate 0.004). At the time of presentation in 2016, the DNA sequencing data was not completed (Sullivan et al. 2016).

Analysis of blood analytes, such as serum LDH, is another possible strategy to identify predictive biomarkers of response. As mentioned above, an elevated LDH is associated with poorer outcomes to high-dose IL-2, although this is true for nearly every therapy for melanoma, and thus is likely more of a prognostic factor than a truly predictive one. A comprehensive analysis of potential blood-based biomarkers identified serum vascular endothelial growth factor (VEGF) and fibronectin as negative predictors of benefit to high-dose IL-2 in patients with melanoma (Sabatino et al. 2009). The IL-2 Select study also captured pretreatment serum in an attempt to validate the predictive value of these biomarkers. These studies are ongoing.

Other Cytokines for Therapy of Metastatic Melanoma

To date, the most efficacious cytokine for the treatment of metastatic melanoma has been high-dose IL-2. IFNα also has activity, but quite modestly in the treatment of metastatic melanoma. Even when combined with anti-PD1 at the maximum tolerated dose of pegylated-IFN-a, clinical activity was at most very modest (Atkins et al. 2018). Other cytokines that have been tested as single agents include: IL-4 (Margolin et al. 1994), IL-6 (Weber et al. 1993, 1994; Sosman et al. 1997), IL-7 (with vaccine) (Rosenberg et al. 2006), IL-12 (Bajetta et al. 1998; Atkins et al. 1997; Gollob et al. 2000; Alatrash et al. 2004), IL-21 (Davis et al. 2007), GM-CSF (Rao et al. 2003), M-CSF (Sanda et al. 1992; Cole et al. 1994; Robertson et al. 2006), and IL-15 (Conlon et al. 2015) (Table 7). None of these has demonstrated significant clinical activity as single agents at the doses tested. There is very limited experience with IL-18; 1 partial responder was noted in a phase I study in 6 patients with metastatic melanoma (Robertson et al. 2006). NKTR-214, the pegylated IL-2 has no single agent activity (see above), but may synergize with anti-PD1 in patients with metastatic melanoma and kidney cancer. More recently, pegylated human recombinant IL-10 or pegilodecakin (AM0010), which stimulates and expands cytotoxic CD8 T cells and inhibits inflammatory CD4 T cells, has been studied as single agent treatment in patients with metastatic solid cancers (Naing et al. 2016). Clinical activity was described in a patient with uveal melanoma and patients with renal cell carcinoma. Pegilodecakin is now being investigated in combination with anti-PD1.

Summary

The first clinical responses with high-dose IL-2 were observed in patients with metastatic melanoma during the mid-1980s. High-dose IL-2 was approved for this disease by the US Food and Drug Administration in 1998. About half of the clinical responses are complete and durable, lasting in excess of 5 years. Lower doses of IL-2, combinations of IL-2 with other cytokines and immune modulators or the use of various other interleukins have not achieved the level of clinical activity of high-dose IL-2. Much has been learned about the management of the side effects of IL-2 and how to safely administer this agent (Schwartzentruber 2001). When administered by properly trained physicians and nurses, this treatment is safe and can be effective in some patients with metastatic melanoma.

Experience with IL-2 based therapy has established that immune modulation does have a role in the treatment of patients with metastatic

Table 7 Clinical responses with cytokines not including IL-2

Author (Reference)	Evaluable patients	Regimen	Response		
			CR	PR	Total (%)
IL-4					
Margolin et al. (1994)	30	IV q 8 h	1	0	1 (3)
IL-6					
Weber et al. (1993)	5	SQ	0	0	0
Weber et al. (1994)	7	IV q 8 h	0	0	0
Sosman et al. (1997)	9	IV	0	0	0
Sosman et al. (1997)	7	CIV × 5 day	0	0	0
IL-7					
Rosenberg et al. (2006)	11	SQ + vaccine	0	0	0
IL-10					
Naing et al. (2016)	4	SQ	0	1	1 (25)
IL-12					
Bajetta et al. (1998)	10	SQ	0	0	0
Atkins et al. (1997)	12	IV	1	0	1 (8)
Gollob et al. (2000)	8	IV	0	0	0
Alatrash et al. (2004)	7	SQ + IFN α SQ	0	1	1 (14)
IL-15					
Conlon et al. (2015)	18	IV	0	2	2 (11)
IL-18					
Robertson et al. (2006)	6	IV	0	1	1 (17)
IL-21					
Davis et al. (2007)	26	IV	1	0	1 (4)
GM-CSF					
Rao et al. (2003)	11	Inhaled	0	0	0
M-CSF					
Sanda et al. (1992)	7	IV Q 8	0	0	0
Cole et al. (1994)	7	CIV	0	0	0

CR complete response, *PR* partial response, *SQ* subcutaneous, *IV* intravenous, *CIV* continuous intravenous, *M-CSF* macrophage colony stimulating factor

melanoma, and this knowledge served as the basis for the development of the current more clinically applicable immune therapies.

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