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Review

Cytokine combinations in immunotherapy for solid tumors: a review

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Abstract. The use of cytokines alone or in combination with other cytokines or cytotoxic drugs has had a profound effect upon widely metastatic disease in many cases. However, despite the encouraging results in early trials, there is much room for improvement. Few responses to these combinations are complete, and toxicity has in some cases been quite severe. Changes in dose, route, or schedule of administration of the drugs, or the development of cytokine analogs may lead to more efficacious and less toxic regimens. In addition, new cytokines such as interleukin(IL)-7 and IL-12 are currently under investigation for potential use in future immunotherapy trials. These prospects and the use of cytokine combinations are promising advances in the treatment of human cancer.

Key words: Cytokines – Immunotherapy – IL-2 – IFN – TNF

Introduction

The recent Food and Drugs Administration (FDA) approval of interleukin(IL)-2 for the treatment of metastatic renal cell carcinoma marks the beginning of a new era in managing patients with disseminated cancer. Clearly, it is now possible to induce the regression of widely metastatic disease in humans by manipulating the immune system with cytokines and other immunomodulators. In addition to IL-2, other cytokines such as tumor necrosis factor (TNF) and IL-4 are currently in various stages of preclinical or clinical testing to determine if they too will be efficacious in the treatment of solid tumors.

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To satisfy phase I requirements, the emphasis of the initial clinical trials involving cytokines has been on the use of single agents (such as IL-2) in the treatment of widely disseminated disease. We will present a brief overview of these trials using single cytokines, but the focus of this review is the use of cytokines in combination with other biological or cytotoxic agents in the treatment of human solid tumors. This is a relatively new approach, and the results of phase I and II trials are just now appearing in the literature.

Cytokines as single agents

IL-2

IL-2 was originally described in 1976 as a protein that supported the growth and proliferation of T lymphocytes [43]. Since then, numerous biological activities have been ascribed to this protein including stimulation of lymphoid cell proliferation; induction of T and NK cell cytotoxicity; and production of numerous other cytokines such as IL-1α, IL-1β, TNFα, TNFβ, IL-6, and interferon (IFN)γ, (reviewed in [65]). In addition, IL-2-stimulated peripheral blood mononuclear cells generate lymphokine-activated killing (LAK), the non-major-histocompatibility-complex-restricted lysis of tumor cells [16].

After a variety of in vitro and in vivo studies suggested that IL-2 would be effective in humans, numerous phase I and II trials involving IL-2 alone or in combination with autologous LAK cells activated ex vivo were conducted. These were recently reviewed by Lotze and Rosenberg [37]. Overall, intravenous (i.v.) IL-2 alone has been found to be effective in approximately 15%–30% of patients with metastatic renal-cell carcinoma (RCC) or melanoma and may be efficacious in the treatment of other tumors as well. The addition of LAK cells to the protocols did not affect the outcome of the patients in the only randomized prospective trial that evaluated the effect of these cells [61]. Despite its efficacy and recent approval by the FDA for the treatment of RCC, IL-2 has not been widely used because

of the severe toxicity that is occasionally associated with its administration [59]. The search for newer approaches to the use of IL-2 that may reduce toxicity will be discussed later in this review.

IL-4

Interleukin-4 was previously called B-cell-stimulatory factor. In humans, IL-4 has been shown to have a variety of effects on both lymphocytes and monocytes including proliferation of B cells, induction of cytolytic T cells, increased growth of tumor-infiltrating lymphocytes isolated from human tumors, and enhanced generation of LAK cells [19].

Recombinant IL-4 has been administered alone to 48 cancer patients; the maximum tolerated dose is approximately 20 μg/kg [36]. In this initial phase I/II trial at the University of Pittsburgh, the administration of IL-4 was associated with the development of vascular leak syndrome, end-organ dysfunction, and weight gain similar to that seen with IL-2. Other toxicites noted were severe nasal congestion and gastric ulceration or inflammation in a small number of patients. No responses were observed in the 48 patients (23 had RCC or melanoma).

IL-6

Like IL-2 and IL-4, IL-6 plays a role in vitro in the differentiation of T lymphocytes. IL-6 induces B lymphocytes to secrete immunoglobulins and potentiates the ability of IL-2 to induce LAK activity. In mice, IL-6 administered alone has led to a decrease in the number of micrometastases [46]. Two phase I trials of IL-6 in 23 patients with refractory advanced malignancies have recently been conducted at the National Cancer Institute [78]. The maximum tolerated dose was approximately 30 μg/kg. All patients who received IL-6 experienced fever and chills, and hepatotoxicity and atrial fibrillation prevented the dose being increased in several patients. The clinical response rates in these trials were not published.

TNF

When Carswell and associates first identified TNF and characterized its ability to induce coagulation necrosis in animal tumors, it was immediately recognized that this protein had tremendous therapeutic potential [5]. However, there have been few responses to intravenous administration of TNF in the numerous phase I and II trials conducted. In fact, in nine separate phase II trials, only 2 of 318 patients with a variety of malignancies responsed to TNF treatment [70]. In addition, the toxicity associated with the i.v. administration of TNF has been severe; patients frequently experience fever, chills, nausea, and vomiting as well as dose-limiting hypotension and vascular leak syndrome. Therefore, the intravenous use of TNF alone does not appear to be effective in the management of solid tumors at this time.

IFN

The interferons play a key role in host responses by modulating immune cell function. They are a family of more than 20 proteins produced after stimulation by antigens, viruses, and double-stranded RNA. The cellular actions of IFN include enhancement of monocyte and NK function and antiproliferative effects [30]. It has been suggested that the antitumor effects result principally from two mechanisms: a direct effect on the function or antigenic composition of tumor cells and an indirect effect on immunological effector cells. Over the past decade, numerous clinical trials have documented the efficacy of IFN against a variety of solid tumors. The mean response rates to IFNα in metastatic melanoma (11 trials) and renal cell carcinoma (11 trials) have been 16% [26] and 14% [71] respectively. Responses of up to 45% have been observed in Kaposi's sarcoma [10] and 83% in pancreatic endocrine tumors [11] treated with IFNα. The more favorable responses were observed in patients who had had resection of bulky disease, suggesting a possible role for IFNα postoperatively as adjuvant therapy. IFNγ, on the other hand, does not appear to be as effective as IFNα in the treatment of solid tumors, although response rates as high as 18% have been observed for RCC [56]. IFNβ also has been somewhat disappointing. Although phase I trials suggested that it could be tolerated at higher doses than IFNα, it was not as effective as IFNα [58].

Cytokine combinations in immunotherapy for solid tumors

As in the development of chemotherapeutic regimens for cancer, the antitumor activities of cytokines used alone have stimulated efforts to evaluate the use of these proteins in combination with each other or with other agents. The combined use of cytokines to increase antitumor efficacy is based upon the theories that combining agents that attack neoplastic cells by different mechanisms should increase the antitumor effects in vivo and that toxicity should be lower in patients treated with combinations of agents that have different side-effects. Preclinical studies have demonstrated that such cytokine combinations can be safe and effective in animal models.

IL-2 in combination with other cytokines

TNF. In a variety of preclinical models, low doses of IL-2 and TNF have been shown to be synergistic activators of LAK cells [51]. This combination has also been evaluated in at least two phase I human trials. In a study at the University of Texas M. D. Anderson Cancer Center [80], 16 patients with advanced non-small-cell lung carcinoma were treated for 5 days with continuously infused IL-2 and 25–100 μg m^{-2} day^{-1} intramuscular (i.m.) TNF. Of the 16 patients treated, only one partial response (PR) was observed. In another study, 31 patients with a variety of advanced malignancies were treated with continuously infused IL-2 and up to 120 μg m^{-2} day^{-1} bolus i.v. TNF [47].

Only two PR (1 patient with breast carcinoma and 1 with RCC) were seen in this study. In both studies, hypotension was dose-limiting, and most patients experienced fever, chills, gastrointestinal toxicity, edema, and malaise.

IL-4. Like TNF, IL-4 also has synergistic effects with IL-2 in the generation of LAK [19]. A phase I/II trial was recently initiated at the University of Pittsburgh to evaluate the use of these cytokines [36]. Twenty-eight patients received IL-4 in conjunction with 7.2×10^5 U/kg IL-2, and the maximum tolerated dose for IL-4 appears to be 20 μg/kg. The spectrum and magnitude of the side-effects associated with this regimen were similar to those seen with IL-2 alone. A total of five responses were noted in the 28 patients including one complete response (CR) in a patient with RCC, three PR in patients with melanoma, and one PR in a woman with breast carcinoma.

IFNβ and IFNγ. IFNβ and IFNγ have many effects upon the body's immune system, including up-regulation of cellular adhesion molecules and enhancement of LAK activity. While neither agent has been shown to be efficacious by itself in the treatment of human cancer, these IFN have been tested in combination with IL-2 in two phase I trials. Krigel et al. [29] administered 5×10^6 U/m^2 IL-2 and 10×10^6 U/m^2 IFNβ (both i.v.) to 47 patients. Of 32 evaluable patients, 1 had a PR. In the other study, Paolozzi et al. [53] treated 50 patients in a phase I study with IL-2 and IFNβ administered subcutaneously (s.c.) and i.v. respectively. The maximum tolerated doses were 5×10^6 U/m^2 IL-2 and 2×10^6 U/m^2 IFNβ. Two patients had PR (one with RCC and one with transitional cell carcinoma). Three phase I studies using i.m. IFNγ and i.v. IL-2 have recently been reported [38, 74, 77]. Although each regimen was slightly different, the maximum tolerated dose for IFNγ was 0.25 mg m^{-2} day^{-1} in each case, and the response rates were similar. In the three studies, there were two CR and six PR in 89 evaluable patients. The severity of toxicity in the IFNβ or IFNγ combination trials was equivalent to those seen with high-dose IL-2 alone.

IFNα. Most of the studies of cytokine combinations have used IL-2 with IFNα. The trials using this combination of cytokines in the treatment of RCC were recently reviewed in detail by Stahl et al. [71]. The remission rates in these studies ranged from 0% to 50%, with CR occurring in 5% and PR in 18% of 342 patients; however, the differences in dose and duration of IL-2, dose and route of administration of IFN, and timing of administration of the two cytokines make comparison of these studies difficult. More recent studies have demonstrated the efficacy of IL-2 and IFNα while minimizing toxicity so that treatment could be administered in an outpatient setting [25, 35]. While the majority of these patients had had nephrectomies, Spencer et al. demonstrated that this therapy may also be effective even in those patients with primary RCC in situ [68]. The combination of IL-2 and IFNα has also been evaluated in the treatment of metastatic melanoma. Again, the dose regimens varied among the studies, and the response rates (CR plus PR) ranged from 0% to 33% [3, 31, 60, 79]. Despite these promising results in the treatment of RCC and melanoma, there is still no clear evidence that the combination of IL-2 and IFNα is superior to IL-2 alone.

TNF in combination with IFNγ

Combinations of TNF and other agents have just begun to be tested, and only phase I data are available. In addition to the previously mentioned trials that used both TNF and IL-2, several trials have evaluated the effectiveness of TNF given in conjunction with IFNγ [1, 8, 21, 63, 66]. Hypotension was the dose-limiting toxicity for this combination and its severity was similar to that of TNF administered alone. Although antitumor responses were observed in cancers of the biliary tract and pancreas and in soft-tissue sarcoma, there is no evidence of synergistic efficacy from these trials. Phase II studies are underway to determine whether TNF plus IFNα will be an effective therapy for human cancers.

IFNγ with IFNα or IFNβ

While the use of IL-2 with IFNα has yielded promising results, early trials using simultaneous administration of either IFNα or IFNβ with IFNγ were disappointing. Substantially more toxicity has been reported in two phase I trials using IFNα and IFNγ but there was only one PR in 35 evaluable patients in the two trials combined [6, 50]. Two randomized studies that compared IFNα and IFNγ alone with IFNα plus IFNγ failed to show clinical synergism of the combination in advanced RCC [9, 12]. In addition, studies by Schiller et al. suggested that little clinical benefit would be obtained by administering IFNβ simultaneously with IFNγ [62].

Combinations of cytokines and cytotoxic agents

The combination of cytokines and cytotoxic drugs offers a new approach to more effective therapy for neoplastic diseases. Currently, there is no optimal strategy for combining these agents because of the number of compounds available and the variations in administration such as route, dose, and timing with respect to the other drugs. Such combinations seek to take advantage of different mechanisms of action, different toxicity profiles, and in vivointeractions between the drugs and the host immune system and drug-metabolizing enzymes. For example, IFN appears to potentiate the effects of 5-fluorouracil (5-FU) by increasing serum levels of active 5-FU metabolites. Moreover, IFN can reverse resistance to 5-FU by inhibiting the overexpression of thymidylate synthase [27]. In addition, there is evidence that the synergy between TNF and doxorubicin and etoposide is related to a rapid increase in the specific activity of topoisomerase I and II, which increases DNA strand breakage [27].

Table 1. Trials involving interleukin-2 (IL-2) and cytotoxic drugs

IL-2 dose ($U\ m^{-2}\ day^{-1}$)	Other drugs	Tumor type	Number of patients[a]			CR+PR (%)	Ref.
			Total	CR	PR		
700/12 h	Mitomycin C	Gastro-intestinal	33	0	10	30	2
18×10^6	Dacarbazine	Melanoma	18	2	2	22	64
4×10^6	Dacarbazine and cisplatin	Melanoma	13	1	4	38	55
$6 \times 10^5\ U\ kg^{-1}\ (8\ h)^{-1}$	Cisplatin	Melanoma	27	3	4	26	7
12×10^6	Cisplatin and 5-FU	NSCLC[a] H/N[a]	18 11	0 2	7 4	39 55	75
$(1-3) \times 10^6$	Doxorubicin	Various	15	0	0	0	4
20×10^6	Doxorubicin	Various	16	0	5	31	52

[a] NSCLC, non-small-cell lung carcinoma; H/N, head and neck carcinoma; CR, complete response; PR, partial response

Table 2. Trials using IL-2 and interferon α (IFNα) with chemotherapy for melanoma

IL-2 dose ($MU\ m^{-2}\ day^{-1}$)	IFNα ($MU\ m^{-2}$)	Other drugs	No. of patients	Percentage responding (%)			Ref.
				CR	PR	CR+PR	
3 qd	5 i.m.	Cisplatin, dacarbazine, vinblastine	30	20	37	57	32
1.5 q 8 h	6 s.c.	Cisplatin, dacarbazine, carmustine, tamoxifen	74	15	40	55	57
18 qd	9 s.c.	Cisplatin	20	20	40	60	24
18 qd	3 s.c.	Cisplatin, dacarbazine	12	25	58	83	17

IL-2 with cytotoxic drugs

Because animal studies showed that the combination of IL-2 and cyclophosphamide could act synergistically in the treatment of advanced tumors, this was the first combination of a cytokine and a cytotoxic agent to be tested in humans. At least seven phase I or II trials have been completed [23, 34, 41, 42, 45, 69, 76]. In each, the patients received an i.v. dose of 350 mg/m^2 cyclophosphamide 3 days before bolus i.v. infusions of IL-2 (3.6×10^6–36×10^6 $U\ m^{-2}\ day^{-1}$). The response rates ranged from 5% to 25% for melanoma; however, only 1 of 36 patients with RCC and 1 of 13 with metastatic breast carcinoma had PR in these trials. Other cytotoxic drugs including dacarbazine, cisplatin, mitomycin C, doxorubicin, and 5-FU have been evaluated in combination with IL-2 in phase I/II trials [2, 4, 7, 52, 55, 64, 75]. The results of these studies are summarized in Table 1. While these IL-2 combinations have demonstrated that such regimens can be safely tolerated by patients, larger prospectively randomized trials will be necessary to determine if they are more effective than the use of single agents alone.

IL-2 and IFNα combinations with chemotherapy

The combination of IL-2 and IFNα has also been tried with chemotherapy as a means of treating metastatic melanoma. At least four studies using this combination have thus far been reported [17, 24, 32, 57], and the results are summarized in Table 2. At least 50% of the patients have responded in each study; CR approached 20%. Although the toxicity experienced by patients in these studies was significant, it was not much different from that seen in patients receiving IL-2 alone, except for the slightly increased myelosuppression secondary to the cytotoxic agents. While in these four trials IL-2 and IFNα were given either before or after the chemotherapeutic agents, one additional trial is currently underway at M. D. Anderson Cancer Center in which IL-2 and IFNα are being given simultaneously with cisplatin, vinblastine, and dacarbazine to treat metastatic melanoma. Early results are encouraging, and the combination appears to be an improvement over prior regimens.

Other cytokines and chemotherapy

IFNα. IFNα has also been studied in combination with various chemotherapeutic agents without IL-2. Because of their single-agent activity against RCC, most investigators combined vinblastine and IFNα in clinical trials for treatment of this disease. The results of 11 phase II and III studies were recently reviewed by Stahl et al. [71]. With this combination, the overall response rate was 24% in 324 evaluable patients. It is difficult to draw conclusions from these studies, however, since two randomized trials comparing IFNα alone or in combination with vinblastine yielded contradictory results. Fossa et al. [13] stated that

the IFNα/vinblastine combination resulted in higher response rates (22% versus 7%), but a multicenter trial [48] found that the combination did not improve response. Combination trials of IFN and other cytotoxic agents for RCC are currently underway.

The combination of IFNα and cytotoxic agents has also been evaluated in the treatment of metastatic melanoma. These results were reviewed recently by Garbe et al. [14]. In several studies combining dacarbazine and IFNα in a total of 203 patients, 19 CR and 36 PR were observed for an overall response rate of 27%. Other trials that added cisplatin (78 patients) or vinblastine (27 patients) showed response rates of 21% and 11%, respectively. In addition, IFNα has been tested in conjunction with vindesine (27% CR plus PR) and dacarbazine + 5-FU (66% CR plus PR) in small numbers of patients with promising but preliminary results [14, 44].

IFNα and 5-FU have been administered together with folinic acid in the treatment of metastatic colorectal carcinoma. Eleven studies reviewed by Kreuser et al. used this combination in a total of 302 patients with a median response rate (CR plus PR) of 25%. This is comparable to studies in which 5-FU and folinic acid were used alone [28].

TNF. There have also been several trials in which TNF was administered with chemotherapeutic agents. As mentioned earlier, TNF synergizes with both doxorubicin and etoposide in animals. Clinical trials have been initiated using both of these combinations. Both the TNF/etoposide trial [49] and the TNF/doxorubicin trial [73] have been remarkable for substantial toxicity. Neither trial reported response rates. The combination of TNF and mitomycin C has also been evaluated in a phase I trial; 2 of 15 patients had PR [54]. No dose-limiting toxicity was identified.

Summary and future directions

While the initial interest in and expectations for many cytokines (especially IL-2) were out of proportion to their current clinical usefulness, these proteins have proven to be useful additions to our therapeutic armamentarium in the fight against cancer. In some instances, the use of cytokines alone or in combination with other cytokines or cytotoxic agents has had profound effects upon widely metastatic disease. For example, IL-2 and IFNα in combination with cisplatin and dacarbazine have produced response rates much higher than any observed with either cytokines or cytotoxic drugs alone. Well-focused, randomized prospective trials are now necessary to confirm the higher responses seen with some combinations.

There is, however, much room for improvement. While many of the trials have demonstrated that cytokines may be effective, few of the responses are complete, and most are not long-lasting. In addition, cytokines have proven to be quite toxic. In one study, 60% of the patients receiving IL-2 alone required pressor support for hypotension, and mortality rates were approximately 2% [59]. However, cytokine combinations have in some cases allowed the use of lower doses of the individual agents. These combinations are occasionally less toxic while efficacy is maintained (or increased).

One approach to improving the tolerance and effectiveness of such cytokine therapy is to determine the mechanisms by which cytokines work. This may allow clinicians to construct therapeutic combinations that act synergistically against tumors. Changing the dose, route, or sequence of drug administration may increase efficacy while increasing patient tolerance of the regimen. For example, new approaches, including either the local injection of cytokines directly into the tumor [20, 81] or intra-arterially [33, 39], may increase local concentrations of cytokine at the tumor site while minimizing systemic toxicity.

Understanding cytokine/receptor interaction may also allow the synthesis of cytokine analogs with the parent cytokine's ability to fight tumors but without their toxicity. For example, we have previously shown that IL-2 analogs that preferentially bind the intermediate-affinity IL-2 receptor retain the ability to generate LAK activity [18] with up to an 85% reduction in the production of secondary cytokines such as IL-1β, TNFα, TNFβ, and IFNγ (submitted for publication), the actions of which are thought to be responsible for the toxicity associated with the administration of IL-2. Also, IL-2 has been chemically modified for clinical use by the addition of polyethylene glycol [22]. It is hoped that such modified agents will have increased half-lives, decreased toxicity, and enhanced efficacy. Further modifications will probably be made to the IL-2 molecule to increase its usefulness in immunotherapy.

Other cytokines are currently being evaluated for potential use in future immunotherapy trials. IL-7, IL-10, and the recently characterized IL-12 can all induce of LAK activity in vitro [15, 67, 72]. In addition, IL-10 induces this oncolytic activity with dramatically less production of two mediators of cytokine-induced toxicity, TNF and IFNγ [67]. New uses for previously characterized cytokines, such as the use of IL-1 as an adjunct in attempts to induce specific antitumor immunity, may also become apparent [40]. These cytokines and others yet to be discovered may prove to be valuable tools in the treatment of human tumors.

As we have shown, significant improvements have been made in experimental cancer therapies by combining cytokines together or with cytotoxic agents to enhance their action and to reduce the doses required to achieve antitumor effects. Currently, cytokines have an established though limited role in the treatment of malignant disease. We anxiously await the expected discovery of improved regimens that optimize the pleiotropic effects of cytokines in the fight against cancer.

References

1. Abbruzzese JL, Levin B, Ajani JA (1989) Phase I trial of recombinant human γ-IFN and recombinant human TNF in patients with advanced gastrointestinal cancer. Cancer Res 49: 4057
2. Arinaga S, Karimine N, Takamuku K, Nanbura S, Inoue H, Abe R (1992) Correlation of eosinophilia with clinical response in patients with advanced carcinoma treated with low dose recombinant IL-2 and mitomycin C. Cancer Immunol Immunother 35: 246

3. Budd GT, Osgood B, Barnes B, Botett JM, Finke J, Medendorp SV (1989) Phase I clinical trial of IL-2 and α-IFN: toxicity and immunologic effects. Cancer Res 49: 6432
4. Bukowski RM, Sergi JS, Budd GT, Murthy S, Tubbs R, Gibson V (1991) Phase I trial of continuous infusion IL-2 and doxorubicin in patients with refractory malignancies. J Immunother 10: 432
5. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B (1975) An endotoxin induced serum factor which causes necrosis of tumors. Proc Natl Acad Sci USA 72: 3666
6. Creagan E, Loprinzi CL, Ahmann DL (1988) A phase I/II trial of the combination of recombinant leukocyte A IFN and recombinant human IFN-γ in patients with metastatic malignant melanoma. Cancer 62: 2472
7. Demchak PA, Mier JW, Robert NJ, O'Brien K, Gould JA, Atkins MB (1991) IL-2 and high dose cisplatin in patients with metastatic melanoma: a pilot study. J Clin Oncol 9: 1821
8. Demetri G, Spriggs D, Herman M (1989) A phase I trial of recombinant human TNF and IFN-γ: effects of combination cytokine administration in vivo. J Clin Oncol 7: 1545
9. DeMulder PH, Debruyne FMJ, von Oosterom A (1991) EORTC randomized phase II study of recombinant IFN-α and recombinant α and γ in advanced renal cell carcinoma. Proc Am Soc Clin Oncol 10: 166
10. Dewit R, Boucher CAB, Veenhof KHN (1988) Clinical and virological effects of high dose recombinant IFN-α in disseminated AIDS-related Kaposi's sarcoma. Lancet 2: 1214
11. Eriksson B, Oberg K, Alm G (1987) Treatment of malignant endocrine pancreatic tumors with human leukocyte interferon. Cancer Treat Res 71: 31
12. Foon K, Doroshow J, Bonueur E (1988) A prospective randomized trial of α–2B IFN/γ-IFN or the combination in advanced metastatic renal cell carcinoma. J Biol Response Mod 7: 540
13. Fossa S, Cavalli F, Otto U (1988) Randomized study of roferon-A with or without vinblastine in advanced or metastatic renal cell carcinoma. Proc Am Soc Clin Oncol 7: 118
14. Garbe C, Kreuser E-D, Zouboulis CC, Stadler R, Orfanos CE (1992) Combined treatment of metastatic melanoma with interferons and cytotoxic drugs. Semin Oncol 19: 63
15. Gately MK, Wolitzky AG, Quinn PM, Chizzonite R (1992) Regulation of human cytolytic lymphocyte responses by IL-12. Cell Immunol 143: 127
16. Grimm EA, Mazumber A, Zhang HZ, Rosenberg SA (1982) The lymphokine-activated killer cell phenomenon: lysis of resistant and fresh solid tumor cells by IL-2 activated autologous human peripheral blood lymphocytes. J Exp Med 155: 1823
17. Hamblin T, Davies B, Saddulah S (1991) A phase II study of the treatment of metastatic malignant melanoma with a combination of dacarbazine, cisplatin, IL-2, and α-IFN. Proc Am Soc Clin Oncol 10: 294
18. Heaton KM, Ju G, Morris DK, Delisio K, Bailon P, Grimm EA (1993) Characterization of lymphokine activated killing by human peripheral blood mononuclear cells stimulated with IL-2 analogs specific for the intermediate affinity IL-2 receptor. Cell Immunol (in press).
19. Higuchi CM, Thompson JA, Lindgren CG, Gillis S, Widner MB, Fefer A (1989) Induction by interleukin 4 of lymphokine-activated killer activity in human lymphocytes preactivated by interleukin 2 in vivo or in vitro. Cancer Res 49: 6487
20. Huland E, Huland H (1989) Local continuous high dose IL-2: a new therapeutic model for the treatment of advanced bladder cancer. Cancer Res 49: 5469
21. Jakubowski A, Larchian W, Starnes F (1988) Phase I trial of intravenous TNF plus IFN-γ in patients with advanced cancer. Proc Am Soc Clin Oncol 7: 164
22. Katre NV, Knauf MJ, Laird WJ (1987) Chemical modification of recombinant IL-2 by polyethylene glycol increases in potency in the murine Meth A sarcoma model. Proc Natl Acad Sci USA 84: 1487
23. Keslitz JE, Wong GY, Welte K (1988) Phase I trial of recombinant IL-2 and cyclophosphamide: augmentation of cellular immunity and T-cell mitogenic response with long term administration of recombinant IL-2. J Biol Response Mod 7: 457
24. Khayat D, Borel C, Antoine E (1992) Highly active chemoimmunotherapy (cisplatin, IL-2, α–IFN) in the treatment of metastatic malignant melanoma. Proc Am Soc Clin Oncol 11: 353
25. Kirchner H, Korfer A, Palmer PA, Evers P, de Riese W, Knuver-Hopf J (1990) Subcutaneous IL-2 and IFNα-2b in patients with metastatic renal cell carcinoma: the German experience. Mol Biother 2: 145
26. Kirkwood JM, Ernstoff MS (1991) Cutaneous melanoma: In: Biologic therapy of cancer. Lippincott, Philadelphia, p 311
27. Kreuser E-D, Wadler S, Thiel E (1992) Interactions between cytokines and cytotoxic drugs: putative molecular mechanisms in experimental hematology and oncology. Semin Oncol 19: 1
28. Kreuser E-D, Hilgenfeld RU, Matthias M, Hoksch B, Bower C, Oldenkott B (1992) A phase II trial of IFNα-2b with folinic acid and 5-FU administered by 4 hour infusion in metastatic colorectal cancer. Semin Oncol 19: 57
29. Krigel RL, Padavia-Shaller KA, Rudolph AR, Litwin S, Konrad M, Bradley EC, Comis RL (1988) A phase I study of recombinant IL-2 plus recombinant β-interferon. Cancer Res 48: 3875
30. Kurzrock R, Talpaz M, Gutterman JU (1991) Interferons α,β,γ: basic principles and preclinical studies. In: Biologic therapy of cancer. Lippincott, Philadelphia, p 247
31. Lee KH, Talpaz M, Rothberg JM, Murray JL, Papadopoulos N, Plager C (1989) Concomitant administration of recombinant human IL-2 and recombinant IFNα-2a in cancer patients: a phase I study. J Clin Oncol 7: 1726
32. Legha S, Plager, Ring S (1992) A phase II study of biochemotherapy using IL-2 and IFNα-2a in combination with cisplatin, vinblastine, and DTIC in patients with metastatic melanoma. Proc Am Soc Clin Oncol 11: 343
33. Lienhard D, Ewalenko P, Delmotte J-J, Renard N, Lejeune FJ (1992) High dose recombinant TNFα in combination with IFNγ and melphalan in isolation perfusion of the limbs for melanoma and sarcoma. J Clin Oncol 10: 52
34. Lindemann A, Hoffken K, Schmidt RE, Diehl V, Kloke O, Gamm H (1989) A phase II study of low dose cyclophosphamide and recombinant IL-2 in metastatic renal cell carcinoma and malignant melanoma. Cancer Immunol Immunother 28: 275
35. Lipton A, Harvey H, Givant E, Hopper K, Lawler J, Matthews Y, et al (1993) Il-2 and IFNα-2a outpatient therapy for metastatic renal cell carcinoma. J Immunother 13: 122
36. Lotze MT (1992) The treatment of patients with melanoma using interleukin 2, IL-4, and tumor infiltrating lymphocytes. Human Gene Ther 3: 167
37. Lotze MT, Rosenberg SA (1991) Interleukin 2: clinical applications: In: Biologic therapy of cancer. Lippincott, Philadelphia, p 159
38. Margolin KA, Doroshow JH, Akman SA, Leong LA, Morgan RJ, Raschko J (1992) Phase I trial of IL-2 plus γ-interferon. J Immunother 11: 50
39. Mavligit G, Zukwiski A, Wallace S (1990) Tumor regression after hepatic arterial infusion of recombinant TNF in patients with colon carcinoma metastatic to the liver. Proc Am Soc Clin Oncol 9: 118
40. McCune CS, Marquis DM (1990) IL-1 as an adjuvant for active specific immunotherapy in a murine tumor model. Cancer Res 50: 1212
41. Mitchell MS (1992) Chemotherapy in combination with biomodulation: a 5 year experience with cyclophosphamide and IL-2. Semin Oncol 19: 80.
42. Mitchell MS, Kempf RA, Havel W (1988) Effectiveness and tolerability of low dose cyclophosphamide and low dose intravenous IL-2 in disseminated melanoma. J Clin Oncol 6: 409
43. Morgan DA, Ruscetti FW, Gallo RG (1976) Selective in vitro growth of T-lymphocytes from normal bone marrows. Science 193: 1007
44. Mulcher NH, Schrafford-Koops H, Sleijfer DT (1991) IFN and DTIC in the treatment of disseminated malignant melanoma. Proc Am Soc Clin Oncol 10: 1020
45. Mule JJ, Rosenberg SA (1991) Combination cytokine therapy: experimental and clinical trials: In: Biologic therapy of cancer. Lippincott, Philadelphia, p 393

46. Mule JJ, McIntosh JK, Jablons DM, Rosenberg SA (1990) In vivo administration of recombinant IL-6 mediates tumor regression in mice. J Exp Med 17: 629
47. Negrier MS, Pourreau CN, Palmer PA, Panchere JY, Mercatello A, Viens P (1992) Phase I trial of recombinant IL-2 followed by recombinant TNF in patients with metastatic cancer. J Immunother 11: 93
48. Neidhart JA, Anderson SA, Harris JE (1991) Vinblastine fails to improve response of renal cancer to IFN-α n1: high response rate in patients with pulmonary metastasis. J Clin Oncol 9: 832
49. Orr D, Oldham R, Levis M, Bertoli L, Birch R (1989) Phase I study of the sequence administration of etoposide and recombinant TNF in patients with advanced malignancy. Proc Am Soc Clin Oncol 8: 741
50. Osaulo S, Jansen R, Naipal A (1989) In vivo effects of combination treatment with recombinant IFN-γ and α in metastatic melanoma. Int J Cancer 43: 1001
51. Owen-SchaubLB, Gutterman JU, Grimm EA (1988) Synergy of TNF and IL-2 in the activation of human cytotoxic lymphocytes: effect of TNF and IL-2 in the generation of human LAK cell cytotoxicity. Cancer Res 48: 788
52. Paciucci PA, Bekesi JG, Ryder RS, Odchimar R, Chahinian PA, Holland JF (1991) Immunotherapy with IL-2 by constant infusion and weekly doxorubicin. Am J Clin Oncol 14: 341
53. Paolozzi F, Zamkoff K, Doyle M (1989) Phase I trial of recombinant IL-2 and recombinant β-IFN in refractory neoplastic diseases. J Biol Response Mod 8: 122
54. Raymonds J, Logan M, Ernstoff M (1990) Phase I study of TNF combined with mitomycin C in cancer patients. Proc Am Soc Clin Oncol 9: 286
55. Redman BG, Flaherty L, Chou T-H, Nakeff A, Pillote K, Kaplan J (1991) Sequential dacarbazine/cisplatin and IL-2 in metastatic melanoma: immunological effects of therapy. J Immunother 10: 147
56. Renal Cancer Study Group (1987) Phase II study of recombinant IFN.γ on renal cell carcinoma. Cancer 60: 929
57. Richards JM, Mehta N, Rammig K, Skosey P (1992) Sequential chemoimmunotherapy in the treatment of metastatic melanoma. J Clin Oncol 10: 1338
58. Rinehart J, Makpeis L, Young D (1986) Phase I/II trial of human recombinant β-interferon serine in patients with renal cell carcinoma. Cancer Res 46: 5364
59. Rosenberg SA, Lotze MT, Yang JC, Aebersold PM, Linehan WM, Seipp CA, White DE (1989) Experience with the use of high-dose interleukin 2 in the treatment of 652 cancer patients. Ann Surg 210: 474
60. Rosenberg SA, Lotze MT, Yang JC, Linehan WM, Seipp CA, Calabro S (1989) Combination therapy with IL-2 and α-IFN for the treatment of patients with advanced cancer. J Clin Oncol 7: 1863
61. Rubin JT, Lotze MT (1993) Adoptive cellular immunotherapy of cancer: In: Biologic approaches to cancer treatment. McGraw-Hill, New York, p 379
62. Schiller JH, Willson JK, Bittner G (1986) Antiproliferative effects of interferons on human melanoma cells in the human colony-forming assay. J Interferon Res 6: 615
63. Schiller JH, Alberti DB, Arzoomanian RZ (1990) Phase I trial of combination of recombinant γ-IFN and recombinant TNFα. Proc Am Cancer Res 31: 183
64. Shiloni E, Poullart P, Janssens J, Splinter T, Di Peri T, Symann M, et al (1989) Sequential dacarbazine chemotherapy followed by recombinant IL-2 in metastatic melanoma: a pilot multicentre phase I/II study. Eur J Cancer 25: S45
65. Smith KA (1988) Interleukin 2: inception, impact, and implications. Science 240: 1169
66. Smith JW, Urba WJ, Clark JW, Longo DL, Farrell M, Creekmore SP, et al (1991) Phase I evaluation of recombinant TNF given in combination with recombinant IFN-γ. J Immunother 10: 355
67. Spagnoli GC, Juretic A, Schultz-Thater E, Dellabona P, Filgueria L, Horig H (1993) On the relative roles of IL-2 and IL-10 in the generation of LAK cell activity. Cell Immunol 146: 391
68. Spencer WF, Linehan WM, Walther MM, Haas GP, Lotze MT (1992) Immunotherapy with IL-2 and α-IFN in patients with metastatic renal cell carcinoma with in situ primary cancers: a pilot study. J Urol 147: 24
69. Spicer DV, Kelley A, Herman R, Dean G, Stevenson L, Mitchell MS (1992) Low dose recombinant IL-2 and low dose cyclophosphamide in metastatic breast cancer. Cancer Immunol Immunother 34: 424
70. Spriggs DR, Yates SW (1992) Cancer chemotherapy-experiences with TNF administration in humans: In: Tumor necrosis factors: the molecules and their emerging role in medicine. Raven Press, New York, p 383
71. Stahl M, Wilke H-J, Seeber S, Schmoll HJ (1992) Cytokines and cytotoxic agents in renal cell carcinoma: a review. Semin Oncol 19: 70
72. Stotter H, Custer MC, Bolton ES, Gwedez L, Lotze MT (1991) IL-7 induces LAK cell activity and is regulated by IL-4. J Immunol 146: 150
73. Taylor C, Hersh E, Plezia P (1989) A phase I and pharmacokinetic study of recombinant TNF and doxorubicin. Proc Am Soc Clin Oncol 8: 183
74. Taylor CW, Chase EM, Whitehead RP, Rinehart JJ, Neidhart JA, Gonzales R (1992) A SWOG phase I study of the sequential combination of recombinant IFN-γ and recombinant IL-2 in patients with cancer. J Immunother 11: 176
75. Valone FH, Gandura DR, Deisseroth AB, Perez EA, Rayner A, Aronson FR (1991) IL-2, cisplatin, and 5-FU for patients with non-small cell lung and head/neck carcinomas. J Immunother 10: 207
76. Verdi CJ, Taylor CW, Croghan MK, Dalke P, Meyskens FL, Hersh EM (1992) Phase I study of low dose cyclophosphamide and recombinant IL-2 for the treatment of advanced cancer. J Immunother 11: 286
77. Viens P, Blaise D, Stoppa AM, Brandely M, Baume D, Olive D (1992) Il-2 in association with increasing doses of IFN-γ in patients with advanced cancer. J Immunother 11: 218
78. Weber J, Rosenberg SA (1993) Phase I studies of the pharmacokinetics, toxicities, and biologic effects of IL-6 in patients with refractory advanced malignancies. J Immunother 13: 74
79. Whitehead RP, Figlin R, Citton ML, Pfile J, Moldauer N, Patel D (1993) A phase II trial of concomitant human IL-2 and IFNα-2a in patients with disseminated malignant melanoma. J Immunother 13: 117
80. Yang SC, Owen SL, Mendigurea RA, Grimm EA, Hong WK, Roth JA (1990) Combination immunotherapy for non-small cell lung cancer. Results with IL-2 and TNFα. J Thorac Cardiovasc Surg 99: 8
81. Yasumoto K, Miyazaki K, Nagashima A (1987) Induction of LAK cells by intrapleural instillations of recombinant IL-2 in patients with malignant pleurisy due to lung cancer. Cancer Res 47: 2184