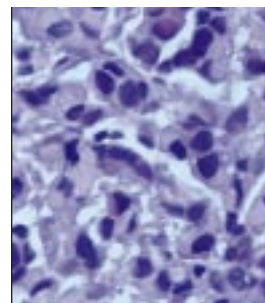


STRATEGIES FOR COMBINING CHEMOTHERAPY AND BIOTHERAPY IN MELANOMA

Antonio C. Buzaid, MD

From the Sociedade Beneficente De Senhoras Hospital Sírio-Libanês, Centro de Oncologia, São Paulo, Brazil.



Introduction

Cutaneous malignant melanoma is an increasingly common clinical problem. Estimates indicate that in 1999, a total of 44,200 new cases of melanoma were diagnosed.¹ Early primary melanoma is cured in most cases, but once the disease becomes metastatic to distant organs, it is almost always fatal. The treatment of metastatic melanoma remains unsatisfactory, with patient survival dictated primarily by the pace of the disease.² Of all the currently employed treatment modalities, cisplatin-based regimens combined with biologic agents such as interferon alfa (IFN- α) and interleukin-2 (IL-2), referred to as "biochemotherapy" or "chemoimmunotherapy," have attained the highest clinical response. The cellular and molecular mechanisms that underlie the apparently greater efficacy of biochemotherapy regimens are unknown, but a sizable body of preclinical data has suggested several possible mechanisms for this observed synergy in human melanoma therapy. This article provides an overview of the clinical studies of systemic therapy in the treatment of advanced cutaneous melanoma, with a focus on biochemotherapy trials. The experimental data on chemotherapy/biologic therapy interactions are also discussed.

Clinical Studies

Chemotherapy

The most active chemotherapeutic drugs in the treatment of

advanced melanoma include dacarbazine (DTIC), cisplatin, the 2-chloroethyl-nitrosoureas (carmustine, lomustine, and fotemustine), vinca alkaloids (vincristine and vinblastine), the taxanes (paclitaxel and docetaxel), and the new agent temozolomide. Observed overall response rates to these single agents have been in the range of 10% to 20%, with complete remissions (CRs) observed in less than 5% of patients.² Combination chemotherapy, including well-known regimens such as cisplatin/vinblastine/DTIC (CVD) and cisplatin/carmustine/DTIC/tamoxifen (Dartmouth regimen), have produced responses in approximately 20% to 40% of patients, but durable CRs have been rare.² Phase III studies comparing combination chemotherapy with single-agent dacarbazine showed only a slight increase in response favoring the chemotherapy combination but no improvement in survival.²

Biologic Therapy

The disappointing results observed with chemotherapy alone shifted the attention of many investigators to the biologic agents. IFN- α and IL-2 have been the most extensively studied biologic agents, with overall response rates in advanced melanoma in the range of 10% to 20%.² Despite the low overall response rates, approximately 3% to 6% of the patients treated with biologics have experienced durable remission.³ Based on preclinical data that suggested a synergistic interaction between IFN- α and IL-2, these biologics have been combined in various clinical

The author is a member of the speakers' bureau for Chiron Therapeutics, Hoffman-LaRoche Inc, and Schering-Plough Corp, and he is an advisor to Bristol-Myers Squibb Co.

studies of patients with advanced melanoma. Overall response rates have ranged from 10% to 41%, averaging 20%.³ The combination of IL-2 plus IFN- α does not appear to have a higher response rate compared with IL-2 alone.⁴

Biochemotherapy

The combination of cytokines, particularly IL-2 and IFN- α with chemotherapy has been the focus of intense investigation by several groups, primarily during the last five years. Such combinations are referred to as "biochemotherapy" by M.D. Anderson Cancer Center (MDACC) investigators and as "chemoimmunotherapy" by others.⁵

Chemotherapy Combined With IFN- α

Interferon alfa has been evaluated in combination with many single-agent chemotherapy drugs as well as with multidrug chemotherapy regimens. Results of phase II studies of IFN- α with DTIC, cisplatin, vinca alkaloids, and nitrosoureas showed activity similar to that of single-agent chemotherapy alone, suggesting a lack of benefit when they were added to these single chemotherapeutic agents.² Four phase III randomized trials comparing chemotherapy alone or combined with IFN- α have used single-agent DTIC as the cytotoxic agent.² Similarly, in phase II

studies that combined IFN- α with the Dartmouth regimen or with CVD as employed at MDACC, response rates were comparable to those of chemotherapy alone.² Collectively, these studies indicated that IFN- α does not add significantly to the response rate, response duration, or survival of combination chemotherapy or DTIC alone.

Chemotherapy Combined With Interleukin-2

The addition of IL-2 to chemotherapeutic agents has been reported by a number of investigators. The most thoroughly evaluated combination, IL-2 plus DTIC, has yielded response rates of 13% to

Table 1. — Selected Phase II Studies of Platinum-Based Chemotherapy in Combination With IL-2 Plus IFN- α for Melanoma

IL-2 Study	Regimen	Number of Patients	Complete Response (%)	Partial Response (%)	Overall Response (%)
Intravenous:					
Richards et al ⁸	CBDT/IL-2/IFN- α	83	15	40	55
Antoine et al ⁹	C/IL-2/IFN- α	129	10	39	49
Legha et al ^{10,11}	Alternating CVD-BIO	39	5	28	33
	Sequential CVD/BIO	30	30	43	73
	Sequential BIO/CVD	30	17	30	47
	Concurrent CVD + BIO	52	21	43	64
Subcutaneous:					
Ron et al ¹²	Carboplatin/DTIC/IL-2(sq)/IFN- α (sq)	16	0	38	38
Atzpodien et al ¹³	Carboplatin/DTIC/IL-2(sq)/IFN- α (sq)	40	7.5	27.5	35
Thompson et al ¹⁴	CBDT/IL-2(sq)/IFN- α (sq)	53	19	23	42
CBDT = cisplatin/BCNU/DTIC/tamoxifen C = cisplatin IL-2 = interleukin-2 IFN- α = interferon alfa CVD = cisplatin/DTIC/vinblastine BIO = IFN- α + IL-2 sq = subcutaneous					

33% (mean, 25%) — not clearly superior to DTIC alone and definitely more toxic than DTIC alone.² IL-2 has also been studied in phase II trials in combination with regimens containing cisplatin and has produced better results than IL-2 plus DTIC, with response rates in the range of 37% to 42%.² The results, while not confirmed in randomized studies, suggested that the interaction of cisplatin with IL-2 might be important and clinically relevant.

Phase II Studies of Cisplatin-Based Chemotherapy With Interleukin-2 Plus IFN- α

Following initial encouraging reports,⁶ several groups examined the use of cytotoxic agents, especially cisplatin-based regimens in combination with IL-2 plus IFN- α to treat patients with advanced melanoma.⁷⁻¹⁴ These results seemed superior to any previously published biochemotherapy regimens (Table 1). The treatment schema of select biochemotherapy regimens is shown in Fig 1.

In most biochemotherapy studies, IL-2 has been administered by the intravenous route (Table 1).⁸⁻¹¹ Richards et al⁷ of the University of Chicago utilized the Dartmouth regimen with the addition of IL-2 and IFN- α . The overall response rate in 42 patients was 57%. In their most recent update with 83 assessable patients, the overall response rate was 55% and the CR rate was 15%.⁸ Of note, there was a 10% progression-free survival beyond 4 years, indicating the possibility of cure with this type of

therapy. Antoine et al⁹ reported the results of the Salpêtrière Hospital experience with cisplatin, IL-2, and IFN- α and observed an overall response rate of 49% among 129 assessable patients.

Investigators from MDACC have conducted a series of sequential phase II trials integrating IFN- α and IL-2 with the CVD regimen; these were designed to explore the effects of different schedules for the chemotherapy and biotherapy.⁵ In the first study, biotherapy and CVD were alternated every 6 weeks to avoid the possibility of interference by chemotherapy with the immunologic effects of biotherapy. Patients were randomly assigned to receive either CVD followed by biotherapy or vice versa. The two regimens produced similar results. The

combined response rate of 33% in the two groups was not superior to previous experience with CVD alone. In the next treatment strategy, influenced by the encouraging preliminary results of the Richards group with a sequential biochemotherapy strategy, the biotherapy and CVD were given one immediately after the other, following the sequential design of Richards and colleagues. This second study, referred to as "sequential biochemotherapy," randomized patients to two arms. Half of the patients received CVD followed immediately by biotherapy. One week later they received a course of biotherapy, then CVD, then biotherapy. The other half of the patients received the opposite sequence starting with the biotherapy/CVD/biotherapy course, and 1 week later they

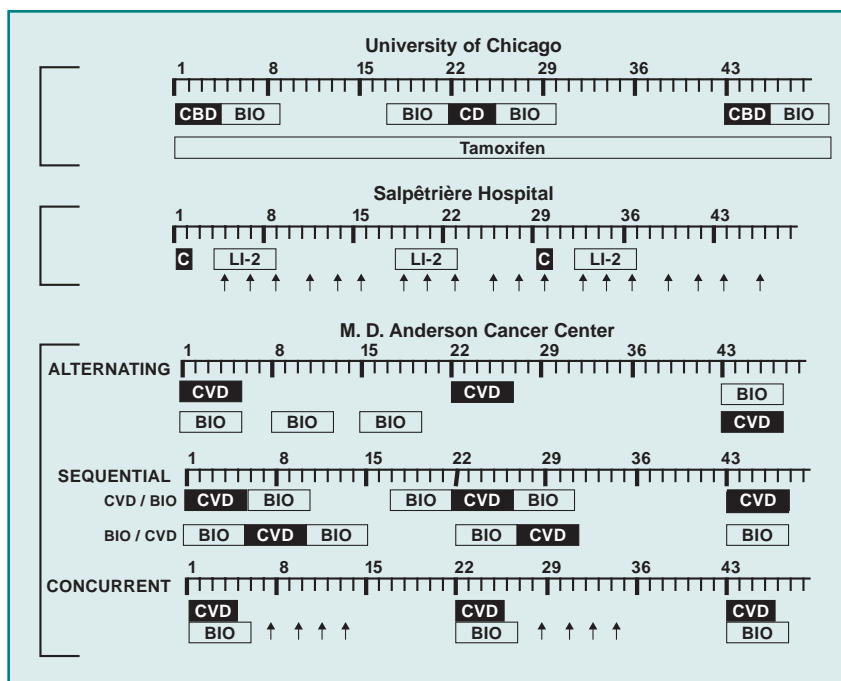


Fig 1. — Treatment schema of select biochemotherapy regimens. C = cisplatin, B = BCNU, D = dacarbazine, V = vinblastine, BIO = interleukin-2 plus interferon alpha, arrow = interferon alpha only.

received biotherapy followed immediately by CVD. Compared with CVD alone, the CVD/biotherapy regimen produced a higher response rate (66% vs 40%), progression-free survival (8 vs 4 months), and overall survival (12 vs 9 months). CVD/biotherapy was also superior to biotherapy/CVD on the basis of response rate (66% vs 50%) and progression-free survival (8 vs 7 months) but not overall survival. Furthermore, among the 31 patients treated with the CVD/biotherapy sequence, 10 achieved a CR compared with only 3 of the 30 patients treated with the biotherapy/CVD sequence, and all 7 durable CRs occurred in the CVD/biotherapy sequence.

In the most recent study, "concurrent biochemotherapy" was used, with the biotherapy and administered the CVD at the same time to reduce the delivery time from 10 to 5 days.¹⁰ The overall response rate of 62% was also superior to that of CVD alone and

approximately half of the 20% CRs remained in complete remission. These MDACC phase II studies suggest that while temporally separated alternating biochemotherapy does not produce additive clinical activity, sequential biochemotherapy using the CVD/biotherapy sequence seemed to produce superior results when compared with CVD alone or with the reverse sequential biochemotherapy sequence. Concurrent biochemotherapy also appeared to be superior to CVD alone and was more convenient and less toxic than the sequential regimen.

Experience with biochemotherapy using IL-2 by the subcutaneous route is more limited (Table 1).¹²⁻¹⁴ Overall, the preliminary results are promising but appear slightly inferior to the intravenous IL-2-based studies. Further study of outpatient biochemotherapy is needed, as reductions in toxicity and cost are also important considerations.

Phase III Studies of Cisplatin-Based Chemotherapy With Interleukin-2 Plus IFN- α

To date, the results of only four randomized studies have been reported (Table 2).¹⁵⁻¹⁸ The European Organization for Research and Treatment of Cancer (EORTC) study compared the Salpêtrière hospital regimen with biotherapy alone.¹⁵ In this study, 138 patients (126 assessable) were randomized to IL-2 plus IFN- α alone vs cisplatin followed by IL-2 plus IFN- α . The biochemotherapy regimen produced a statistically superior response rate ($P=0.04$), but there was no difference in time to progression or in overall survival.

Johnston et al¹⁶ reported the results of a randomized study comparing the Dartmouth regimen vs the Dartmouth regimen preceded by IL-2 (days 2 to 0) and followed by IFN- α (days 1 to 3). Thirty patients were randomized to the chemotherapy regimen and 35 to

Table 2. — Phase III Studies of Biochemotherapy with Chemotherapy, Biotherapy Alone, or Chemobiotherapy

Author	Regimen	Number of Patients	Complete Response (%)	Partial Response (%)	Overall Response (%)
Keilholz et al ¹⁵	C/BIO	60	5	28	33
	BIO	66	6	12	18
Johnston et al ¹⁶	CDBT/BIO	35	3	20	23
	CDBT	30	0	27	27
Dorval et al ¹⁷	C/BIO	52	4	21	25
	C/IL-2 alone	49	6	10	16
Rosenberg et al ¹⁸	CDT/BIO	50	6	38	44
	CDT	52	8	19	27

BIO = IL-2 plus IFN- α
 C = cisplatin
 D = DTIC
 B = BCNU
 T = tamoxifen

the biochemotherapy arm. There was no difference in response rate, time to progression, or survival. The lower response rate in this study may relate to the sequence of administration of the IL-2 that preceded rather than followed the chemotherapy component.

Dorval et al¹⁷ reported the results of a randomized trial that compared the Salpêtrière regimen of cisplatin followed by IL-2 plus IFN vs cisplatin followed by IL-2 without IFN, thus testing the value of IFN in this biochemotherapy combination. Although the response with the biochemotherapy regimen was higher than with cisplatin plus IL-2 alone (25% vs 16%), this difference was not significant. Furthermore, there was no difference in time to progression or in survival.

More recently, Rosenberg et al¹⁸ reported the results of a phase III study conducted at the National Cancer Institute. Patients were randomized to receive either cisplatin, dacarbazine, and tamoxifen or cisplatin, dacarbazine, and tamoxifen followed by IL-2 plus IFN. In 52 patients treated with chemotherapy alone, there were 14 objective responses (27%), including 4 complete. In 50 patients treated with biochemotherapy, there were 22 objective responses (44%) ($P=0.071$), including 3 complete. There was a trend toward a survival advantage for patients receiving the chemotherapy alone ($P=0.052$; median survival of 15.8 months compared with 10.7 months). It is unusual in oncology, however, that a regimen with a higher response

rate and without a high mortality rate would have an adverse impact on survival. This unusual survival finding is most likely due to the small number of patients and probable imbalances between the treatment arms.

A common denominator to the four randomized studies reported to date is the limited number of patients leading to a lack of power to detect small but clinically meaningful differences that could exist in the long-term survival impact of biochemotherapy. Furthermore, in some studies, the investigators launched a phase III trial without even testing their regimen in a phase II study, which is critical not only to determine the activity of that particular combination but to provide clinical experience with a more complex regimen. There are currently two large, randomized phase III studies designed to determine whether or not biochemotherapy increases long-term survival compared with chemotherapy alone. One study that was

recently completed at MDACC compared the CVD regimen with the sequential biochemotherapy program (Fig 2). The other is being conducted under the auspices of the Southwest Oncology Group and the Eastern Cooperative Oncology Group and compares the CVD regimen with a slightly modified concurrent biochemotherapy (Fig 3).

Potential Mechanisms of Interaction Between Biologics and Chemotherapy

The clinical rationale of the majority of biochemotherapy studies so far has been based primarily on two principles: the independent activity of each component and the apparent absence of cross-resistance between biologics and cytotoxic agents when used against melanoma.⁶ Although most clinical trials have been designed with these empirical principles in mind, a sizable body of preclinical data

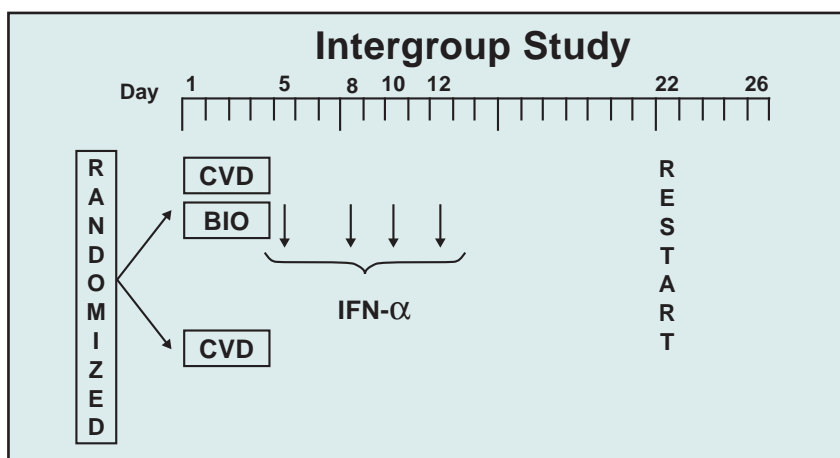


Fig 2. — Schema of the Intergroup phase III study comparing concurrent biochemotherapy vs CVD chemotherapy alone. CVD = cisplatin, vinblastine, DTIC; BIO = interleukin-2/interferon-alfa.

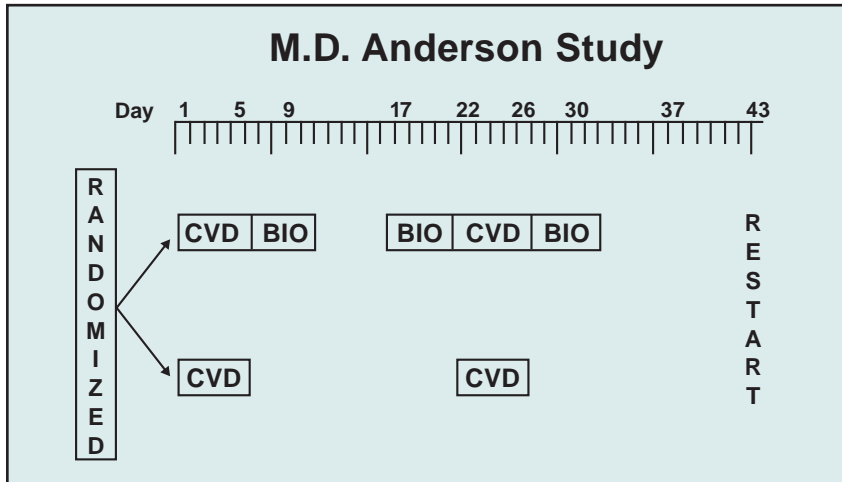


Fig 3. — Schema of the M.D. Anderson Cancer Center phase III study comparing sequential biochemotherapy vs CVD chemotherapy alone. CVD = cisplatin, vinblastine, DTIC; BIO = interleukin-2/interferon-alfa.

has demonstrated synergism between the biologics and cytotoxic agents. Based on these data, several hypotheses have been proposed. Simplistically, these can be divided into two major categories: (1) the chemotherapy enhances the antitumor effect of the biotherapy and (2) the biotherapy enhances the antitumor effect of the chemotherapy. As the evidence for these two hypotheses is explored in detail, the importance of drug scheduling in the observed anticancer effect will be highlighted. Despite the wealth of data supporting both hypotheses, the precise mechanism of anticancer effect of biochemotherapy regimens remains to be determined.⁶

Evidence That Chemotherapy Enhances Biotherapy

While selective enhancement of the immune response by cytotoxic agents has been widely documented, a common denominator of

the most active biochemotherapy regimens has been the presence of cisplatin in the chemotherapeutic component. Indeed, most of the data concerning the effects of chemotherapeutic agents on immune function focus on cisplatin. For other chemotherapy agents, the data are more limited.

Cisplatin and Immune Function

In the early 1970s, Rosenberg,¹⁹ who initially reported on the anticancer activity of cisplatin, suggested that cisplatin's antitumor effect could result in part from its effect on the immune system, apparently because it enhanced the antigenicity of the tumor. Since then, a number of studies have been reported on the effects of chemotherapeutic agents, particularly cisplatin, on the immune system. These preclinical and clinical data have suggested that cisplatin affects the immune system in

important direct and indirect ways that may be relevant to the efficacy of biochemotherapy programs.

Effect on Monocyte/Macrophage Function

— As early as 1980, Kleinerman et al²⁰ reported that cisplatin enhanced monocyte-mediated cytotoxicity in vitro. Confirming these findings, Sodhi et al^{21,22} also found that cisplatin increased the production of hydrogen peroxide, superoxide anion, and IL-1 in murine macrophages in vitro. Among clinical studies, Kleinerman et al²³ investigated spontaneous monocyte-mediated cytotoxicity in 34 patients with various malignancies treated with cisplatin-based regimens and in 31 normal controls. Compared to the normal controls, cancer patients showed significantly decreased spontaneous monocyte cytotoxicity (43% vs 7%). In 7 patients, the monocyte cytotoxicity observed during 6 cycles of chemotherapy increased by at least three- to four-fold between the 3rd and 5th cycles. This study suggested that cancer patients have depressed monocyte function and that cisplatin-based chemotherapy seems to enhance this activity. In contrast, using non-cisplatin-based regimens, Lower and Baughman²⁴ evaluated the production of hydrogen peroxide by human peripheral blood monocytes and showed that chemotherapy caused a reversible impairment of monocyte function in breast and lung cancer patients. Although the in vitro studies demonstrated a direct cisplatin effect, the in vivo and clinical data did not show conclusively how cisplatin activates the macrophages.

Natural Killer/Lymphokine-Activated Killer Cells — Cisplatin has also been shown to enhance natural killer (NK) cell activity in vitro and in vivo^{21,25} and to stimulate lymphokine-activated killer (LAK) cells, apparently by the induction of IL-1 and tumor necrosis factor (TNF).²⁶ The effects of the dose of cisplatin on the host's capacity to kill tumor cells were studied by Crum²⁷ in a rat sarcoma tumor model. Crum observed that low doses of cisplatin increased the activity of circulating lymphocytes that mediate antitumor activity, while high doses of cisplatin suppressed lymphocyte activity. Although the clinical data on this research area are more limited, they agree with those of the in vitro and

in vivo studies. For example, Arinaga et al²⁸ found that a single dose of cisplatin given to cancer patients increased the ability of peripheral blood mononuclear cells to produce LAK activity upon stimulation with IL-2 but had no effect on NK activity. Other investigators have observed similar results.⁶

T-cell Function — The effects of cisplatin on T-cell function have been examined in relatively few preclinical investigations. Tsuda et al²⁹ evaluated the effects of cisplatin on T-cell suppressor activity in 15 ovarian cancer patients and showed that cisplatin did not affect NK cell activity but selectively decreased suppressor cell activity up to 7 days after treatment.

Immunophenotyping revealed that the CD56+/CD16+ and CD8+/CD11+ T-cell population decreased significantly, while CD4+/2H4+ T cells increased significantly after therapy. The magnitude of the decrease in suppressor activity was modest, and the clinical significance of this finding is unclear.

Tumor-Cell Susceptibility to Effector Cell Killing — Various investigators have shown that pretreatment of tumor cells with low concentrations of cisplatin increased the susceptibility of the tumor cells to both NK and LAK killing.³⁰⁻³³ Furthermore, Bernsen et al³⁴ demonstrated in a murine model that the characteristics of the tumor cells affected the results of the interaction of cisplatin and IL-2. They found that two different murine tumors with comparable sensitivity to either IL-2 or cisplatin alone had different sensitivities to combined therapy (sequential treatment with cisplatin and then IL-2), which suggested that cisplatin could make the tumor cells either more sensitive or less sensitive to the IL-2 antitumor effect, regardless of the effect of IL-2 as a single agent.

Other Cytotoxic Agents — Only a few other cytotoxic agents have immune-stimulating effects. Cyclophosphamide, like cisplatin, augments immune

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response by decreasing T-cell suppressor cell activity.^{35,36} In addition, Watanabe et al³⁷ showed that when human colon cancer cells were exposed to low concentrations of fluorouracil (5-FU), they became more susceptible to LAK killing. These studies demonstrated that other cytotoxic agents might also have important immunomodulatory effects. These effects are minor, however, and no clinical relevance has yet been shown for the use of low-dose, immunostimulating cytotoxic drug therapies.

In summary, the predominant interaction between the biologic agents and cisplatin may be the immunostimulation of host effector cells by both, leading to enhanced tumor-cell kill relative to that achieved with cisplatin plus the other cytotoxic agents but without effector-cell stimulation (Fig 4). Potential mechanisms of effector-cell-mediated cytotoxicity include NK/LAK-type cytotoxicity, antibody-dependent cellular cytotoxicity, cytokine-mediated damage (eg, by IL-1 and TNF- α), and DNA damage induced by effector-cell production of reactive oxygen/nitrogen intermediates.⁶ All of these killing mechanisms are potentially inducible in the effector cells by the components of the biochemotherapy.

Evidence That Biotherapy Enhances Chemotherapeutic Effect

A large number of in vitro and in vivo studies have shown that IFN can have an additive or synergistic effect with many cytotoxic agents, including cisplatin, doxorubi-

cin, 5-FU, and vinblastine.³⁸ Despite this wealth of in vitro data, a direct interaction between IFN and chemotherapeutic agents probably does not play an important role in the biochemotherapy regimens' anticancer activity; most studies in which IFN- α alone was combined with single-agent chemotherapy have shown response rates similar to those of chemotherapy alone. Unlike IFN, IL-2 has not shown important interactions with cytotoxic agents in vitro because its primary effect is on effector cells and requires an intact host to demonstrate anticancer activity. IL-1, the cytokine that initiates the immune-effector and cytotoxicity cascades and possesses independent antitumor activity in some cases, has also been shown to act in a synergistic manner against various tumor cells in combination with many cytotoxic agents.³⁹ In one study,⁴⁰ some human ovarian cancer cell lines treated with the combination of IL-1 α and cisplatin showed enhanced cisplatin accumulation in cells and inhibited DNA repair. Likewise, TNF- α has been found to have in vitro synergistic interaction with various cytotoxic drugs including carmustine, doxorubicin, cisplatin, etoposide, and melphalan,^{41,42} although the mechanism for the synergism has not been well defined. The clinical relevance of these in vitro findings remains to be determined, but it is conceivable that, during biochemotherapy treatment, IL-2 induction of secondary cytokines such as IL-1 and TNF- α could directly enhance the antitumor effect of cytotoxic agents.

The effects of activation of tumor-infiltrating immune cells on the antitumor effect of chemotherapeutic drugs have been evaluated in relatively few studies. The original studies by Braunschweiger et al⁴³ provided important contributions to this complex area of research. In a murine model bearing a squamous cell carcinoma cell line, the investigators showed that cisplatin and IL-1 α were synergistic in vivo. They also showed that dexamethasone, but not indomethacin, could abrogate the synergism, which suggested that prostaglandins were not involved and that the immune system (either macrophages or lymphocytes) or endothelial cells could be playing an important role in this interaction.⁴⁴ To test for the participation of macrophages, the authors evaluated the effects of cisplatin and IL-1 α using a co-culture model composed of cisplatin-pretreated tumor cells and tumor-infiltrating macrophages. When untreated tumor cells were exposed to host cells with or without IL-1 α stimulation, little cytotoxicity was seen. When cisplatin-pretreated tumor cells were exposed to IL-1 α , marked tumor-cell killing was observed only in the presence of tumor-infiltrating macrophages. In addition, this synergistic interaction could be abrogated by catalase, which suggested that IL-1-induced release of hydrogen peroxide from the macrophages was responsible for the synergism.⁴⁴ These experiments highlighted the importance of host cells and the key role of oxidant stress in enhancing cisplatin-induced tumor cytotoxicity in this tumor model.

Another potential mechanism by which biotherapy may enhance the antitumor effect of chemotherapeutic drugs is via the production of nitric oxide (NO). NO has been shown to kill many types of cancer cells in vitro, including the melanoma cell line A375,⁴⁵ and the effect is synergistic with the cytotoxic activity of cisplatin.⁴⁶ Various cell types have been shown to produce NO in animal and human systems. Although the role of NO as a mediator of macrophage antitumor activity in rodent systems is well established, significant NO production by stimulated human monocytes and macrophages has not been universally observed.⁶ Human endothelial cells, in contrast, have been shown to produce NO in both a constitutive fashion and after stimulation with inflam-

matory cytokines.⁴⁷ Further, Chang et al⁴⁸ demonstrated that endothelial cells derived from murine tumors produced significantly more NO in response to IL-1 α and IFN-gamma than did normal endothelium. Last, several studies have shown that tumor cells, including melanoma, can be induced to make NO and/or express NO synthase upon stimulation with cytokines or cytotoxic agents in vitro and seemingly exhibit growth arrest or cell death as a consequence.⁶ Braunschweiger et al⁴⁶ have recently shown that NO is synergistic with cisplatin in human melanoma cell lines resistant to cisplatin. Human IL-2 therapy is well known to induce NO production, and in one small study,⁴⁹ the degree of NO production correlated with response

to IL-2-based therapy. Thus, NO, induced in tumor cells or produced by macrophages and/or endothelium by IL-2, secondary cytokines (such as TNF or IFN-gamma, and cisplatin may make an important contribution to the clinical effect of biochemotherapy by enhancing chemotherapy effect against melanoma cells through direct tumor toxicity and potentiation of cisplatin DNA damage.

Therefore, in addition to the hypothesis that cytotoxic agents and biologics stimulate immune-effector cells to produce the enhanced tumor killing activity of biochemotherapy, further preclinical data pointed to two other hypotheses concerning the mechanism of biochemotherapy against advanced melanoma. First, biotherapy may enhance the effect of the cytotoxic agents by stimulating tumor-infiltrating macrophages to increase oxidant stress, thereby inhibiting repair of DNA damage caused by the cytotoxic agents (Fig 5). Second, the biotherapy (and chemotherapy) may induce the production of NO by tumor-infiltrating macrophages, endothelial cells, or the tumor cells themselves, which would enhance the chemotherapeutic effect by direct cellular toxicity and possibly by inhibiting repair of DNA damage (Fig 6).

Scheduling of Biotherapy and Chemotherapy

In most studies of the combination of biologics with chemotherapeutic drugs, the chemothera-

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py dosing preceded administration of the biologics. However, the effect of schedule on the synergistic interaction between biologics and chemotherapy drugs has been studied systematically by only a few investigators. Gauny et al⁵⁰ evaluated the combination of IL-2 with various cytotoxic agents in two murine models: Meth A sarcoma and B16 melanoma. In the Meth A sarcoma model, cisplatin, bleomycin, and doxorubicin demonstrated enhanced effects when administered before or concurrent with IL-2. No efficacy was observed, however, when IL-2 was given first. In addition, a synergistic effect was seen only with cisplatin. In the B16 model, when cyclophosphamide was given before to or concurrent with IL-2,

it produced complete regression in existent tumors and prevented tumor establishment. When IL-2 was administered before cyclophosphamide, only tumor growth delay was seen. However, neither cisplatin nor 5-FU had any efficacy when combined with IL-2 in this model.⁵⁰ Also using a B16 melanoma model, Rinehart et al⁵¹ showed that IL-2 combined with cyclophosphamide, etoposide, and cisplatin chemotherapy was more effective than chemotherapy alone only when chemotherapy was given before IL-2. Formelli et al,⁵² studying the combination of immune lymphocyte infusion and cisplatin in mice bearing YC8 lymphoma cells, observed that the antitumor effect was stronger when cisplatin was administered

on day 5 and immune lymphocytes on day 7 compared with administration of immune lymphocytes on day 5 and cisplatin on day 12. In contrast, Lumsden et al⁵³ found no schedule dependence in the effectiveness of doxorubicin and IL-2 against colonic adenocarcinoma in a rat model.

The importance of timing has also been studied for cytotoxic drugs and IL-1 α . Nakamura et al⁵⁴ investigated the interaction of IL-1 α given in combination with a number of cytotoxic agents, including mitomycin C, doxorubicin, cisplatin, cyclophosphamide, and 5-FU, in two murine models: Meth A sarcoma and colon 26 adenocarcinoma. IL-1 α was administered one day before, concurrent with, or one day after administration of the cytotoxic drug. Treatment was started on day 1 or 7 after intradermal transplantation of the tumor. In the Meth A sarcoma model, with drug administration starting on day 7, enhancement of the antitumor effect was observed for all drugs, but it was more marked with doxorubicin and 5-FU. The timing of doxorubicin administration did not significantly affect the number of cures, but more cures were observed when 5-FU was administered either concurrent with or one day before IL-1. In the colon 26 model, timing was not a factor when the drugs were administered on day 1 of tumor transplantation. When the drugs were administered starting on day 7 of tumor transplantation, however, cisplatin was significantly more effective when

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administered concurrently with IL-1. No difference was observed when cisplatin was given one day before or one day after IL-1 α . Detailed *in vivo* studies concerning the schedule dependence of the synergism between IL-1 and cytotoxic drugs were also performed using RIF-1 murine model.^{43,44,55} The investigators observed that mitomycin C acted synergistically with IL-1 when it was administered 1 hour before IL-1, whereas cisplatin was synergistic with IL-1 when administered 6 hours before and up to 2 hours after IL-1. Chang et al,⁵⁶ however, observed opposite results from both carboplatin and cisplatin treatment with IL-1 α . They found that the cytotoxic synergy was significant only when IL-1 α was administered 4 to 12 hours before the platinum compound. There is no obvious explanation for these differing results as both groups were using similar animal models.

Despite some conflicting reports, the results of most animal studies have suggested that the efficacy of combined cytotoxic/biologic treatment is higher when the cytotoxic therapy is administered either before or concurrent with the biologic agent. These findings agree with the results of the biochemotherapy studies at MDACC, which suggested that concurrent biochemotherapy is an active combination and that the CVD/biotherapy combination seems to be more effective than the biotherapy/CVD combination. Further studies designed to evaluate the importance of schedule are needed.

Correlative Clinical Studies

Despite extensive *in vitro* and *in vivo* studies on the interaction between biologics and cytotoxic drugs, the synergistic mechanism for most interactions has not been clearly elucidated. A smaller number of clinically correlated mechanistic studies are relevant to the biochemotherapy programs in melanoma. Primarily using peripheral blood, these studies have been conducted with patients undergoing biochemotherapy for advanced melanoma. They have focused on the effect of chemotherapy on the immune system, the effect of immune function and biotherapy on cytotoxic drug activity, and the biological correlates of activity with response. However, no consistent finding or correlation has been found among the limited number of studies reported.⁶ A randomized trial is currently being conducted at MDACC that compares sequential biochemotherapy with CVD alone. In this randomized trial, a series of laboratory studies designed to evaluate DNA repair in peripheral mononuclear cells and tumor cells, as well as various immunologic studies that focus on the monocyte/macrophage and nitric oxide, are being performed in both treatment arms to discover the potential mechanisms of interaction between chemotherapy and immunotherapy. However, it is anticipated that these clinical studies will provide primarily circumstantial evidence to support or refute this hypothesis and that *in vitro* and animal models will be necessary to accurately elucidate the mechanisms of interac-

tion between biologics and chemotherapeutic drugs.

Conclusions

Biochemotherapy with cisplatin-based chemotherapy combined with IL-2 and IFN- α holds promise as a new therapy for advanced melanoma. Preliminary results from several centers have suggested a dramatic improvement in response rates, complete responses, and duration of response. These early results may foretell a significant improvement in survival compared with currently available therapies. The mechanism of biochemotherapy's enhanced activity against melanoma is not known, but a large body of preclinical data points to some biological interactions that may be involved. Indirect evidence supports a key role for the monocyte/macrophage and its soluble mediators, especially NO and reactive oxygen intermediates, in the anticancer effects observed. Hypotheses for the mechanism of action of this therapy include (1) chemotherapeutic enhancement of biotherapy-induced immune effects, (2) biotherapy-stimulated tumor-associated macrophage production of reactive oxygen intermediates that would affect the repair of chemotherapy-induced DNA damage, and (3) enhancement of the chemotherapeutic effect by induction of local NO production, which would also inhibit the repair of chemotherapy-induced DNA damage. Further basic and applied research is necessary to elucidate the biological

mechanism behind the observed clinical effects. Only a better understanding of the antitumor mechanisms will allow us to improve the current results of biochemotherapy for patients with advanced melanoma.

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