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NDA 21-240

Histamine Dihydrochloride for Injection

BRIEFING DOCUMENT FOR

ONCOLOGIC DRUGS ADVISORY COMMITTEE

December 13, 2000

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SYNOPSIS

Development of Histamine Dihydrochloride as an Adjunct to Interleukin-2 in the Treatment of Patients with Metastatic Melanoma

Introduction

Histamine Dihydrochloride for Injection has been developed by Maxim Pharmaceuticals, Inc. as an adjunct to interleukin-2 (IL-2) for the combination treatment of patients with Stage IV melanoma. Histamine combined with a subcutaneously (SC) administered lower dose regimen of interleukin-2 (aldesleukin) demonstrated a significant survival benefit for those melanoma patients that had liver metastases and the combination treatment was associated with lower toxicity and an improved quality of life.

In support of an indication for histamine dihydrochloride as an adjunct to IL-2 for metatstatic melanoma patients with liver metastases, Phase 1, 2 and 3 clinical trials of histamine dihydrochloride in advanced Stage IV melanoma patients have been completed. A New Drug Application (NDA) for Histamine Dihydrochloride for Injection was submitted to the FDA on July 18, 2000. The NDA was designated priority review status. Combination therapy of histamine dihydrochloride and IL-2 is intended to treat patients with a serious, life-threatening illness and provides a meaningful therapeutic advance to patients over existing treatments. Therefore, the NDA was given accelerated approval status under the provisions of 21 CFR § 314 Subpart H, Accelerated Approval of New Drugs for Serious or Life-Threatening Illness. Histamine dihydrochloride for injection is not registered in any other country, nor has regulatory approval ever been withdrawn in any other country.

Melanoma

Metastatic melanoma remains a frustrating and almost invariably fatal disease. As yet no single agent chemotherapeutic or immunotherapeutic regimen has shown a significant affect on survival in large randomized trials. Combination chemotherapy or biochemotherapy regimens may improve response rates, but not survival.[1] Median survival in most studies ranges from 7 to 11 months with 5-year survival rates under 4%. In a recent longitudinal review of 1,521 patients followed at a single institution, median survival and 5-year survival rates were found to be essentially unchanged over a 22-year period[2]**.** Several groups have investigated the long-term outcome of melanoma patients treated with chemotherapy or interferons between 1982 and 1995. These investigations included patients enrolled in Phase 2 chemotherapy trials[1,3], interferon trials[4], clinical trials with combination treatments[5], or regardless of intent-to-treat[6]. The conclusion of these reports was that the survival of patients with stage IV melanoma has remained unchanged over the last 25 years and was not influenced by treatment. The median survival ranged between 4 and 7.5 months, and the 5-year survival between 2% and 6%.

Interleukin-2 (IL-2) is a lymphokine produced by normal T lymphocytes and was initially described in 1976. It is a member of the class of polypeptide autocrine and paracrine growth factors that regulate immune responses. It is central to both cellular and humoral arms of the immune system and regulates lymphocyte proliferation and differentiation and is produced by the CD4+ T-cells, and a subset of CD8+ cells. IL-2 can induce proliferation of T-cells and NK cells independently (autocrine) and together with other lymphocyte modulating cytokines. IL-2 is currently approved for the treatment of patients with advanced renal cell carcinoma and metastatic melanoma in the U.S.

Several phase 2 clinical studies have reported that high dose intravenous bolus interleukin-2 (IL-2) has therapeutic activity in metastatic melanoma and renal cell carcinoma, yet the impact on survival has never been evaluated in large randomized phase 3 trials. Immunotherapy with high-dose interleukin-2, has produced durable complete responses in a small percentage of patients and combinations of cisplatin-based chemotherapy and IL-2 based immunotherapy have produced responses in approximately 50% of patients with 10% durable complete responses.[7] Unfortunately, high-dose IL-2 regimens are associated with significant multi-organ system toxicity restricting their use to specialized units capable of providing intensive care.[8] Life-threatening adverse events are not uncommon.

It has been suggested that tumor-induced immunosuppression may be, in part, responsible for the relative lack of consistent patient benefit observed with cytokines such as IL-2 or INF-alpha in several tumor types. One hypothesis that has emerged over the last several years suggests that phagocyte-derived reactive oxygen metabolites (ROM) down-regulate intratumoral lymphocytes rendering them nonresponsive to cytokines or other lymphocyte activating agents. In fact, it has clearly been shown that monocyte or macrophage-derived ROM will irreversibly inhibit specific NK-cell and T- cell functions in response to IL-2 such as cytotoxic activity, proliferation, cytokine production and DNA replication, ultimately leading to apoptosis of both NK and T-cells.

Therefore, a logical therapeutic strategy that incorporates an inhibitor of ROM production and release may protect NK cells and T-cells, at the site of the tumor, and allow for more effective activation and enhancement of NK cells and T-cells by cytokines or other immunomodulating treatments. Hence, the role for histamine. Histamine is a potent inhibitor of ROM production by phagocytic cells such as monocytes, macrophages and neutrophils. The effect is mediated strictly through the H2 receptor and results in disruption of the NADPH-oxidase responsible for ROM production. Histamine synergizes with IL-2, and other cytokines, and allows for more effective activation of NK cells and T-cells. There is extensive literature supporting this hypothesis and the combination use of histamine and cytokines in cancer.

Histamine Dihydrochloride for Injection has been tested in sixteen clinical trials, to date, in combination with IL-2 and/or IFN-á in patients with advanced melanoma, renal cell carcinoma, acute myelogenous leukemia and in hepatitis C. The early clinical results in metastatic melanoma demonstrated the safety and potential efficacy of the combination treatment using histamine dihydrochloride with various regimens of IL-2 and IFN-á. A large randomized phase 3 trial demonstrated that the histamine containing regimen was safe, and

provided a statistically significant improvement in survival for patients with liver metastases compared to patients receiving IL-2 alone.

Phase 3 Study Design (Histamine Dihydrochloride as an Adjunct to Subcutaneous Interleukin-2)

Maxim Pharmaceuticals sponsored a multicenter, randomized, controlled, pivotal phase 3 study to evaluate whether histamine dihydrochloride given as an adjunct to subcutaneous interleukin-2 would improve the duration of survival in adult patients with metastatic melanoma. In the study (Study MP-US-M01, also referred to as M01) patients with histologically proven metastatic melanoma were randomized to receive subcutaneous IL-2 or subcutaneous IL-2 plus histamine. Patients were randomized by a centralized procedure through an independent CRO but not pre-stratified according to any known prognostic factors.

The primary endpoint was survival, an unambiguous gold standard of efficacy, one that has been a regulatory endpoint in traditional drug approval[9], and applied prospectively to the Intent-to-Treat Population (ITT) and the Intent-to-Treat Population with Liver Metastases at baseline (ITT-LM). Secondary endpoints were time to progression, tumor response rate, quality-of-life, and safety.

All patients received a regimen of subcutaneous IL-2, twice daily, plus either histamine dihydrochloride (1 mg by slow subcutaneous infusion, twice daily) or nothing. The IL-2 dose was 9.0 MIU/m^2, BID, on days one and two of weeks one and three of the cycle and 2.0 MIU/m^2, BID, on days one through five of weeks two and four of the six week cycle. Patients were given no treatment in weeks five and six.

Efficacy Results

Three hundred five (305) patients were enrolled at 56 institutions in the US. The first patient was enrolled on July 3, 1997; enrollment continued until March 8, 1999. At the time of data cut-off for survival analysis (March 8, 2000), all patients had a minimum of 12 months follow-up. There were no patients lost at the 12-month follow-up; the survival status of every randomized patient was known on March 8, 2000. The sponsor has agreed with the requirement of the Division of Oncologic Drug Products (DODP) that every patient be followed until death in study M01. The efficacy results (from the March 8, 2000 database) are shown in Table A.

Table A. Overview of Phase 3 Study Results (Study M01)

Efficacy Results	Median Survival (months)		p-value [a]
	IL-2 Alone	IL-2 plus Histamine	
All Randomized Patients with Liver Metastases	5.1	9.4	0.0080
All Randomized Patients	8.2	9.1	0.1255

[a] Log-rank test. p-value adjusted by Holm-Šidák multiple comparison procedure. The raw p-value was 0.0040.

Safety Results

In a phase 1 study in healthy adult volunteers receiving two injections of histamine dihydrochloride daily as a single agent for 14 days, all volunteers experienced at least one adverse event (Maxim study MP-MA-0403). Through the safety data cut-off date of December 10, 1999, data were available for 17 volunteers (8 male, 9 female, mean age 41.7 years). The most common drug related adverse events were transient injection site inflammation (100%), vasodilation (100%), headache (94%), injection site pain (65%), conjunctivitis (59%), injection site reaction (53%), and injection site hypersensitivity (47%). Almost all events were rated as mild; less than 10% of events were described as moderate. There was a single event described as severe - asthenia in a volunteer who received 15 µg/kg. There were no serious adverse events in the phase 1 study. All events resolved within 30-60 minutes without treatment and left no sequelae.

In the phase 3 randomized study the incidence of adverse events was high in both study groups. However, the incidence of adverse events was not greatly different between patients receiving IL-2 alone and patients receiving IL-2 plus histamine dihydrochloride. Similarly, the severity of adverse events was similar between the two groups. Table B summarizes the severity of adverse events in patients receiving IL-2 alone or IL-2 plus histamine regardless of relationships to study drug. The data in Table 3 combines patients in the phase 3 randomized study and an ongoing phase 2 single arm study (Maxim study MP-MA-0103) which increase the number of included patients to 186.

Table B. Incidence of Adverse Events by Severity in Metastatic Melanoma Patients in Maxim Studies MP-US-M01 and MP-MA–0103.

	Mild (grade 1)	Moderate (grade 2)	Severe (grade 3)	Life-Threatening (grade 4)
Histamine Plus IL-2 (N = 186)	**4%**	**39%**	**46%**	**11%**
IL-2 Alone (N = 152)	**3%**	**36%**	**51%**	**11%**

The incidence of adverse events associated with discontinuation on the study was similar in both treatment arms when studies M01 and 0103 are combined. For patients receiving histamine plus IL-2, 30/186 (16.1%) experienced an adverse event associated with study discontinuation whereas in patients receiving IL-2 alone 21/152 (13.8%) experienced adverse events associated with study discontinuation. The most frequent adverse event that was associated with discontinuation was coded to "skin melanoma" and therefore is due to progressive disease.

The incidence of adverse events associated with death was similar in both treatment arms when studies M01 and 0103 are combined. Among patients receiving histamine plus IL-2, 23/186 (12.4%) experienced an adverse event associated with death whereas in patients receiving IL-2 alone 25/152 (16.4%) experienced adverse events associated with death. In nearly all cases the adverse event again coded to "skin melanoma" and therefore was a manifestation of the underlying disease.

An analysis of the safety data showed that incidence of adverse events was slightly greater in the patients receiving histamine dihydrochloride plus IL-2 compared to patients receiving IL-2 alone. However, adverse events attributable to histamine were generally mild and resolved within 60 minutes without treatment. It was concluded that histamine added very little to the toxicity burden of patients receiving SC IL-2 for metastatic melanoma.

Quality-of-Life

Quality-of-Life was evaluated in Study M01 by the use of the Quality of Well-Being Self-Administered (QWB-SA) instrument. The QoL analysis was comprised of two main components: 1) comparison of QoL scores of patients while on study therapy between treatment groups, and 2) integration of QWB-SA scores with survival and comparison of quality-adjusted survival between groups. Of the 305 patients randomized in study M01, 301 (98.7%) completed at least one QWB-SA questionnaire and were included in the analysis.

The addition of an adjunct therapy (namely, histamine dihydrochloride) administered as a slow subcutaneous injection twice daily, five days/week for 4 weeks during a treatment cycle, did not adversely impact QoL. Assessment of the quality-of-life data in study M01, including statistical analysis, leads to the conclusion that patients receiving histamine plus IL-2 will experience a similar QoL compared to patients receiving IL-2 alone. In other words, histamine when used in combination with subcutaneous IL-2 does not cause any degradation in the quality of life. This is especially important when considered in light of the home-based administration for both IL-2 and histamine in this study and the intensive-care hospital based dosing required for high-dose intravenous bolus IL-2.

For the ITT population, the median quality-adjusted survival was 31.3 days longer in the histamine plus IL-2 group compared to the IL-2 alone group (105.6 days vs. 74.3 days, respectively; $p = 0.007$). For the population comprised of all randomized patients with liver metastases at baseline, the median quality-adjusted survival was 50.2 days longer in the histamine plus IL-2 group compared to the IL-2 alone group (113.0 days vs. 62.8 days, respectively; $p = 0.010$).

Treatment with histamine plus IL-2 improves the duration of survival of melanoma patients with liver metastases, with minimal impact to the patient in terms of toxicity or reduced quality-of-life.

Benefit/Risk Assessment

The benefit of the use of Histamine Dihydrochloride for Injection in conjunction with interleukin-2 in the treatment of patients with metastatic melanoma is evidenced by the statistically significant increase in the duration of survival for patients with liver metastases. No other therapy has established a significant survival effect in metastatic melanoma. In study M01 the increase in the median duration of survival was 4.3 months (an 84% increase) for patients receiving histamine plus IL-2 compared to IL-2 alone. This difference was highly significant ($p = 0.0080$).

In the group receiving histamine plus IL-2, the proportion of patients surviving 24 months was greater than would be expected based on historical reports in the literature. In the ITT-LM population, 26% of patients receiving histamine plus IL-2 survived for 24 months compared to 7% of patients receiving IL-2 alone.

The increase in the duration of survival obtained as a result of treatment with histamine plus IL-2 is accomplished with minimal and transient increases in adverse events. The profile of adverse events for patients receiving histamine plus IL-2 compared to those for patients receiving IL-2 alone is not greatly different, although for some expected events the incidence is greater for patients receiving histamine. Vasodilation, headache, injection site reaction, hypotension, dizziness, injection site inflammation, rhinitis, pruritis, palpitation, paresthesia, conjunctivitis, neck pain, and skin disorder were expected and all occurred somewhat more frequently in patients in the histamine plus IL-2 treatment arm compared to those receiving IL-2 alone. Adverse events which may be attributable to histamine are generally mild, transient (resolve without treatment in 30-60 minutes), and leave no sequelae.

Study M01 has demonstrated that histamine plus subcutaneous IL-2 results in a markedly less toxic treatment regimen than the bolus intravenous high dose IL-2 treatment regimen, a regimen which has not been shown to increase survival in randomized trials. Intravenous IL-2 at a dose of 600,000 to 720,000 IU/kg given every 8 hours is extraordinarily toxic. Patients receiving high dose intravenous IL-2 must be hospitalized for the duration of the 14 injection sequence on two occasions (usually five days each, with a nine day rest in between if patients are able to tolerate the entire sequence). Patients with normal cardiovascular, pulmonary, hepatic, and CNS function may experience serious, life threatening or fatal adverse events with high dose IV interleukin-2. The FDA approved labeling for Proleukin® (aldesleukin) states, "Adverse events with high dose IV interleukin-2 are frequent, often serious, and sometimes fatal." The labeling for Proleukin® states that patients were able to tolerate a median of 18 doses (of 28 which would be a full course).

In marked contrast, patients receiving lower dose subcutaneous injections may remain at home and either receive injections from a home-care health professional or may be trained to administer themselves. In the study M01, all injections of IL-2 and histamine were given at home. The availability of a home based regimen for IL-2 and histamine is an extremely important benefit for patients with life threatening metastatic melanoma.

The decision to prescribe any particular medical treatment is of course made by the physician. However, patients with terminal cancer frequently decide for themselves, with advice and counsel from physicians and other health care providers, spouse and family, which course of treatment (if any) will be used. The decision is fundamentally a personal one - to be made within the context of the patient's life experiences, values, and existing co-morbidity. Every patient will have a unique set of life experiences, tolerance for pain and discomfort, and emotional and psychological stability in the face of terminal metastatic melanoma and will view the decision within that context. The physician and the manufacturers of the pharmaceuticals that may be prescribed for treatment and/or palliation are responsible for providing accurate and unbiased information regarding potential benefits and likely risks so that patients can make rational and informed decisions.

Within the setting of terminal metastatic melanoma with liver involvement, the benefit/risk relationship for histamine dihydrochloride given in conjunction with subcutaneous interleukin-2 should be considered highly favorable – the benefits clearly outweigh the risks. There is essentially little or no serious additional risk conferred by the subcutaneous administration of 1 mg histamine dihydrochloride, but the benefit of increased duration of survival may be extremely valuable to patients suffering from a terminal malignant disease. Moreover, while the toxicity of IL-2 is substantial, the benefits obtained from receiving subcutaneous injections of IL-2 at home are far superior to receiving bolus intravenous injections every 8 hours in two five-day intensive-care hospital stays.

Conclusions

No single agent chemotherapeutic or immunotherapeutic, no polychemotherapy or biochemotherapy regimen has demonstrated in large randomized trials a significant prolongation of survival in patients with Stage IV melanoma. Overall the higher response rates observed with certain multidrug regimens have not correlated with prolonged survival and these regimens are invariably quite toxic. It seems reasonable to focus on survival as a primary endpoint and test less toxic regimens which evaluate Quality of Life (QoL) in Stage IV melanoma patients. Such studies may show regimens of lower toxicity to be medically equal to the toxic regimens and to be far superior with respect to maintenance of the patient's QoL. Moreover, containment of costs becomes increasingly important, if not mandatory and these non-toxic treatments will obviously, as outpatient treatments with little requirement for concomitant medication, be cost effective.

The material summarized in this document on the randomized trials combining Histamine Dihydrochloride for Injection with subcutaneous IL-2, performed in the outpatient setting, demonstrated that histamine is an effective adjunct to IL-2 in the treatment of melanoma patients with liver metastases and that it can be administered safely. This is the first large randomized trial, to our knowledge, demonstrating a significant survival benefit, even in a subpopulation, for stage IV melanoma patients.

Based on the consistent and positive benefits documented thus far in the histamine trials, Maxim is seeking approval of Histamine Dihydrochloride for Injection for use as an adjunct in combination with IL-2 for the treatment of adult patients with metastatic melanoma that has metastasized to the liver.

San Diego, California
November 8, 2000

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Abbreviations and Definitions of Terms

AJCC	American Joint Committee on Cancer
ASCO	American Society of Clinical Oncology
BSA	Body Surface Area
CDDP	Cisplatin
CI	Confidence Interval
CR	Complete Response
DODP	Division of Oncologic Drug Products (FDA)
DSMB	Drug Safety Monitoring Board
DTIC	Dacarbazine
EORTC	European Organization for the Research and Treatment of Cancer
EORTC-MCG	EORTC Melanoma Cooperative Group
FDA	Food and Drug Administration
IFNá	Interferon alfa
IL-2	Interleukin-2 (Proluekin®)
IV	Intravenous, Intravenously
KPS	Karnofsky Performance Score
LDH	Lactate Dehydrogenase
LSMeans	Least Square Means
MIU	Million International Units
MR	Minimal Response
NCI	National Cancer Institute
NDA	New Drug Application
NK	Natural Killer (cells)
PR	Partial Response
ROMs	Reactive Oxygen Metabolites
SC (sc)	Subcutaneous, Subcutaneously
SD	Stable Disease or Standard Deviation (depending upon context)
UICC	Union Internationale Contre le Cancer
ULN	Upper Limit of Normal
WHO	World Health Organization

1. Background Information

1.1 Natural History and Existing Therapy for Metastatic Melanoma

Melanoma

Melanoma is a malignant tumor of melanocytes; cells derived from the neural crest. Although most melanomas arise from the skin, they may also arise from mucosal surfaces or at other sites to which melanocytes migrate. It can remain localized or metastasize widely. The American Joint Committee on Cancer and the Union Internationale Contre le Cancer have adopted a four-stage system to categorize patients with melanoma based on disease status. This is summarized in Table 1.

Table 1. Four-Stage System for Classification of Melanoma Adopted by the American Joint Committee on Cancer[9] (AJCC) and Union Internationale Contre le Cancer (UICC)

Stage	Criteria
I	Localized melanoma, ≤ 0.75 mm or Level II[a] Localized melanoma 0.76 – 1.5 mm or Level III
II	Localized melanoma, >1.5 – 4 mm or Level IV
III	Localized melanoma, >4 mm or Level V Metastasis in any regional lymph node(s) and / or in-transit metastasis[b]
IV	Distant metastasis. Metastasis in skin or subcutaneous tissue or lymph nodes beyond the regional lymph node and / or visceral metastasis[b]

[a] Thickness of tumor given as millimeters or Clark's Level (Level II, invading the papillary dermis; Level III, invades to the papillary-reticular dermal interface; Level IV, invades the reticular dermis; Level V, invades the subcutaneous tissue).

[b] In-transit metastasis involves skin or subcutaneous tissue more than 2 cm from the primary tumor not beyond the regional lymph nodes.

Incidence and Prevalence of Melanoma

The projected incidence of malignant melanoma in the United States in 1999 was 44,200 new cases.[10] The incidence of malignant melanoma is rising faster than the incidence of any other malignant tumor. Although this rise is observed particularly with respect to thin melanomas, most of which can be cured by surgery alone, the incidence of high-risk melanomas is also rising. According to SEER Cancer Statistics Review 1999, approximately 4% of patients with melanoma have distant

metastatic disease at the time of diagnosis.[11] The calculated prevalence of advanced malignant melanoma in 1999 was 5,023 patients. On the basis of this information, Histamine Dihydrochloride for Injection was granted Orphan Drug Status by FDA on February 1, 2000.

It is important to note is that melanoma occurs predominantly in young to middle age adults (median 45 years). Therapy that can significantly prolong life or cure stage IV melanoma will do so in many patients in the most productive years of their lives.

Stage IV melanoma, defined by the presence of distant metastasis, has a very poor prognosis, with a median overall survival of 6 to 9 months and a mortality rate greater than 95% at 5 years. Stage IV melanoma that has metastasized to distant visceral sites has a very poor prognosis, regardless of therapy; The American Cancer Society reports a median survival time of approximately 6 months for stage IV melanoma with visceral involvement.[12]

Metastatic melanoma remains a frustrating and almost always fatal disease. As yet no single agent chemotherapy or immunotherapy regimen has provided in randomozed trials a significant affect on survival. Combination chemotherapy or biochemotherapy regimens may improve response rates, but this has not correlated to a survival advantage.[13] Median survival in most clinical studies ranges from 7 to 11 months with 5-year survival rates under 4%. In a recent longitudinal review of 1,521 patients followed at a single institution, median survival and 5-year survival rates were found to be essentially unchanged over a 22-year period.[14] Several groups have investigated the long-term outcome of melanoma patients treated with chemotherapy or interferons between 1982 and 1995. These investigations included patients enrolled in Phase 2 chemotherapy trials,[13,15] interferon trials[16], and clinical trials with combination treatments.[17,18] The conclusion of this report was that the survival of patients with stage IV melanoma [American Joint Committee on Cancer (AJCC)] has remained unchanged over the last 25 years and was not influenced by treatment. The median survival ranged between 4 and 7.5 months, and the 5-year survival between 2% and 6%. Other studies have reported a median survival of 6-12 months primarily depending on the patient-population demographics.[13, 19-23]

Prognostic Factors

The site of distant metastases is believed to be a significant prognostic indicator in stage IV melanoma.[19-24] Distant metastases can be divided into visceral and nonvisceral groups. In the University of Alabama study by Balch et al[25] soft tissue metastases (skin, subcutaneous tissue, lymph nodes) were most common (median survival of 9-12 months). The lung is the next most common site of metastases (solitary lung metastases: median survival is 11.4 months), followed by brain, liver, and bone (median survival of only 2 to 6 months and a 1-year survival rate of 8% to 10%).[23] In melanoma, as in many solid tumors, male sex is associated with poorer prognosis.[19-24] The number of metastatic sites and lactate dehydrogenase (LDH) serum levels are also of prognostic importance. In a case record based study of 631

patients with advanced melanoma Keilholz *et al*. found three pretreatment factors to be significantly associated with survival: 1) performance status, 2) number of metastatic sites, and 3) serum LDH.[26] The dominant prognostic variables apparent from a review of the medical literature using multifactorial analysis are LDH, performance status, number of metastatic sites, anatomic site of the metastasis, and male sex.

Systemic Treatment of Stage IV Melanoma

There is no standard therapy for the treatment of Stage IV melanoma. Surgery and radiation are regarded as palliative treatment only and are suitable for patients with limited disease and at certain sites only. Biochemotherapy has not been demonstrated to have a significant impact on survival.[12,14-16] Dacarbazine (DTIC) is the most popular and most widely used chemotherapeutic agent. It yields response rates between 15 and 25%, with a complete response (CR) rate of about 5% and about 1-2% long term survivors.[13,26,27] Whether DTIC treatment prolongs survival is not known, nor has this question ever been addressed in a randomized phase 3 trial comparing any combination chemotherapy, immunotherapy or biochemotherapy.

Cisplatin (CDDP) may be the second most active drug in metastatic melanoma and polychemotherapy combination regimens such as the BOLD regimen[28] or the CVD regimen[29] have been shown to yield increased response rates without proof of prolonging survival.[13,26] This has recently been demonstrated in a randomized phase 3 trial comparing Vindesine (VDS) + DTIC vs. Cisplatin (CDDP) + Vindesine + DTIC (CVD) , reported by the Scandinavian Melanoma Cooperative Group.[30] The addition of Cisplatin added significant toxicity without an improvement in survival.

In the United States the "Dartmouth Regimen" (cisplatin, BCNU, DTIC and tamoxifen) is a commonly used treatment regimen. It was first reported to produce response rates of 40-50%[31] with subsequent wider usage, response rates have declined to 15-30%.[32] Recently the "Dartmouth Regimen" was reported to have no survival benefit over treatment with DTIC alone in a phase 3 randomized trial in 240 patients.[33]

The use of tamoxifen is not recommended, as there is no proof that the addition of tamoxifen to chemotherapy improves survival in spite of the initial positive outcome of a (small) phase 3 trial.[34] Large Phase 3 cooperative trials annulled the earlier reports of Rusthoven et al[35] and Falkson et al.[36] The large cooperative trials could not find any evidence of activity associated with the addition of Tamoxifen to polychemotherapy (DTIC + Cisplatin + Carmustine),[37] or monochemotherapy with DTIC.[38] In the end all these results lead us to conclude that no combination chemotherapeutic schedule has been proven superior to treatment with DTIC alone.

Immunotherapy

Single agent Interferon alfa (IFNα) therapy and single agent Interleukin-2 (IL-2) treatment yield response rates of about 15% in patients with metastatic melanoma with a limited number of patients experiencing long term responses and long term survival.[39,40] The combination of IFNα and IL-2 has been reported to increase response rates[41,42] but this has not been confirmed in a randomized phase 3 trial comparing IL-2 versus IL-2 + IFNα.[43]

Results obtained in 270 stage IV melanoma patients treated with high dose bolus administration of recombinant human interleukin-2 (rhIL-2) (600,000 IU -720,000 IU/kg every 8 hours) according to the original schedule of the NCI Surgery Branch resulted in the approval of this regimen because of durable responses in a small percentage of patients.[44] Complete responses (CR) occurred in 6% and partial responses (PR) in 10% for an overall response of 16%. Ten out of 12 CRs were still free of disease after 5 or more years of follow up. This report established a role for IL-2 in the treatment of melanoma as one of the very rare treatment options in disseminated melanoma that could result in a durable complete response. In daily practice however, high dose bolus IL-2 has limited clinical use because of its extraordinary toxicity, the requirement that administration must be done in a hospital intensive care setting, and the associated high costs.

Biochemotherapy

High response rates, consistently above 50%, have been reported in a number of phase 2 and phase 3 trials where combinations of cytostatic drugs and cytokines have been tested, such as CDDP + IFNα + IL2;[45] CDDP + Vinblastine + DTIC + IFNα + IL2 in various sequential schedules;[46] DTIC + CDDP + BCNU + IFNα + IL2 + Tamoxifen;[47] Bleomycin + Vincristine + Lomustine + DTIC + IFNα;[48] CDDP + DTIC + Tamoxifen + high dose bolus IL-2.[49] However in spite of these high response rates subsequent phase 3 trials presented a disappointing picture.

In DTIC based studies the addition of IFNα has been reported to increase response rates and survival only in one small trial.[50] Three large phase 3 trials with a total of 701 patients have not found higher response rates or prolonged survival by combining IFNα with DTIC.[38,51,52]

Similarly in IL-2 based studies no benefit was observed when the efficacy of CDDP + IL-2 was compared to CDDP + IL2 + IFNα in 117 patients.[53] Neither was a survival benefit observed in the EORTC-Melanoma Cooperative Group (MCG) trial comparing IL-2 + IFNα with or without CDDP in 138 patients, in spite of a doubling of the response rate in the CDDP containing arm.[54] The only randomized trial that has shown a modest impact on survival (median survival increased from 9 to 11 months) is the MD Anderson sequential biochemotherapy trial vs. chemotherapy (CVD), reported by Buzaid et al. at the ASCO meeting in 2000. Unfortunately, the

biochemotherapy regimen was associated with prohibitive toxicity and prolonged in-hospital stay, and on these grounds is considered an option for very few patients and certainly not one that can be widely administered safely.

In summary, to date, no single agent chemotherapeutic or immunotherapeutic, or polychemotherapy or biochemotherapy regimen has demonstrated a significant prolongation of survival in patients with Stage IV melanoma. Overall the higher response rates observed with multidrug regimens do not correlate with prolonged survival and these regimens are invariably very toxic. It seems reasonable to now focus on survival as a primary endpoint and test less toxic regimens which consider Quality of Life (QoL) in Stage IV melanoma patients. Such studies may show regimens of lower toxicity to be medically equal to the toxic regimens and to be far superior with respect to maintenance of the patient's QoL.

The material summarized in this document on the randomized trial combining Histamine Dihydrochloride for Injection with subcutaneous IL-2, performed in the outpatient setting, demonstrated that histamine is an effective adjunct to IL-2 in the treatment of melanoma patients with liver metastases. This is the first large randomized trial, to our knowledge, demonstrating a significant survival benefit, even in a subpopulation, using a feasible and low toxicity, outpatient treatment option for stage IV melanoma patients

1.2 Other Indications

Maxim Pharmaceuticals is also sponsoring clinical trials of histamine dihydrochloride in acute myeloid leukemia, renal cell carcinoma, and hepatitis C. None of these indications is proposed for marketing at this time under NDA 21-240. Supplemental applications may be submitted in the future to provide for additional indications. All patients receiving histamine either as a single agent or in combination, regardless of indication, are included in the safety evaluation.

1.3 Histamine Dihydrochloride for Injection

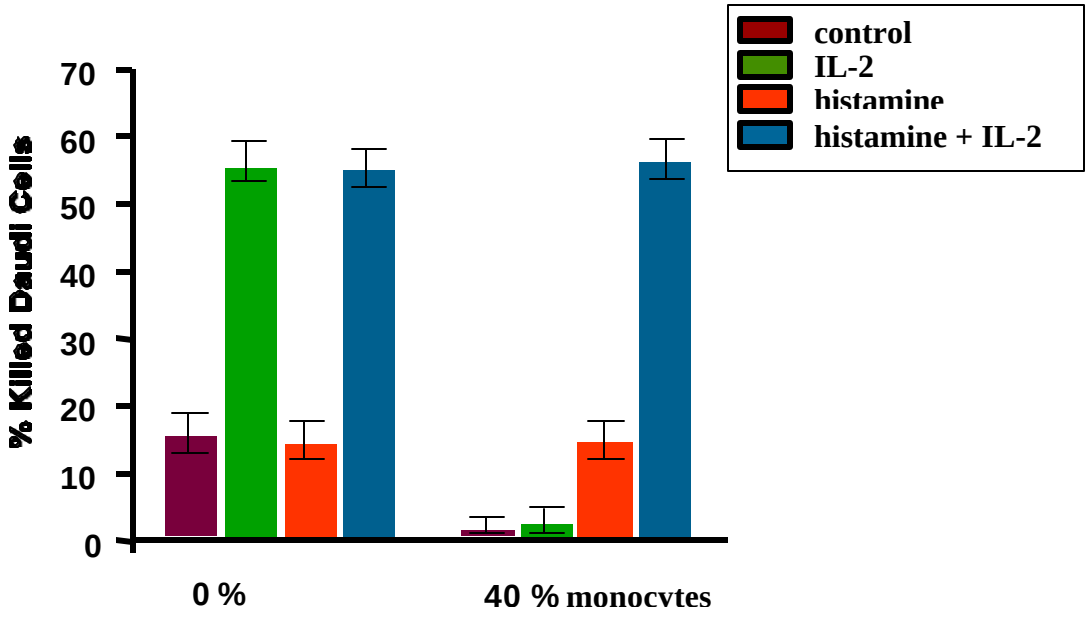
1.3.1 Mechanism of Action and Metabolism

1.3.1.1 Mechanism of Action.

Inhibition of NK and T-Cell Activity by Phagocytes

Several observations have been published suggesting that an increased number of monocytes located in and around tumors leads to a less favorable prognosis for patients with melanoma, breast adenocarcinoma and colorectal carcinoma.[55-57] The phagocytic cells predominately found in and around the tumor are monocytes and macrophages. In a series of elegant experiments, Hellstrand *et al*., have shown that these phagocytic cells strongly inhibit the tumoricidal

activity of Natural Killer (NK) cells and T-cells at the site of the tumor by releasing reactive oxygen metabolites (ROMs). These ROMs irreversibly suppress NK cell and T-cell activation and cytotoxicity at the tumor site leading to anergy and apoptosis.[38,58-60] This appears to explain why IL-2 or IFN-α therapy cannot induce NK and T-cells to kill tumors since the activation is inhibited by ROMs produced by the ever-present phagocytes. An example of one such experiment is highlighted in Figure 1.



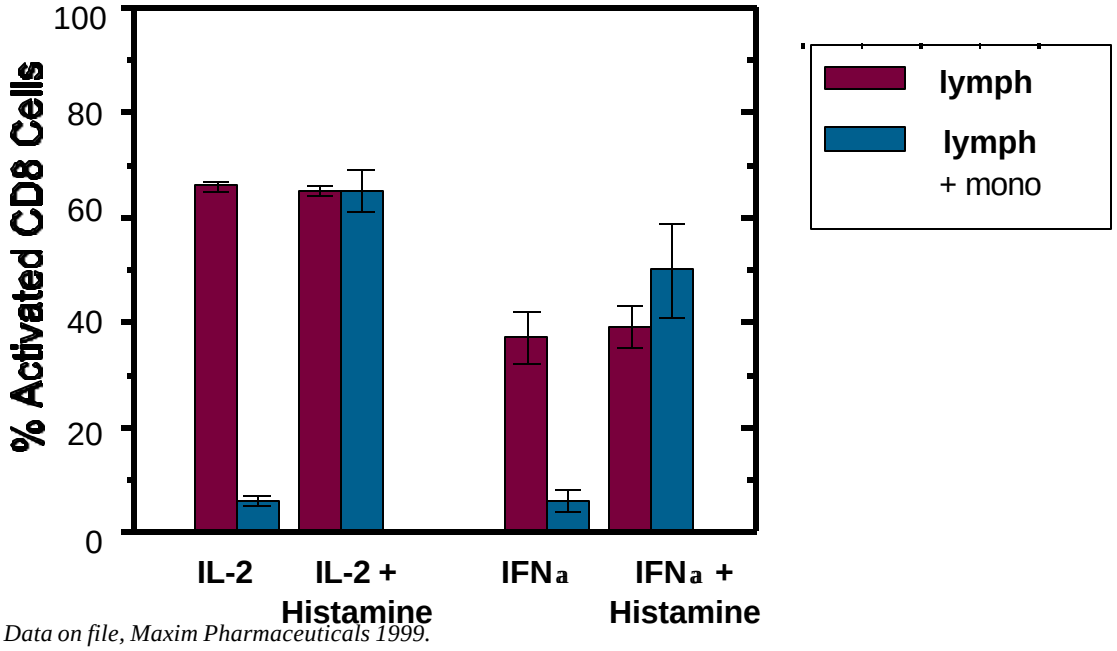
From: Hellstrand et al. J. of Immunol 1990; 145:4365-4370.

Figure 1. Daudi Tumor Cells

Figure 1 shows the ability of NK-cells, when mixed with Daudi tumor cells, to kill up to 20% of the Daudi cells *in vitro* without any cytokine enhancement (purple bar, left panel). When IL-2 is added, there is significant augmentation of this killing activity when monocytes are not present (green bar, left panel). Histamine introduced to NK cells mixed with Daudi cells does not alter the activity of the NK cells in the presence or absence of IL-2 (orange and blue bar, left panel). However, when monocytes are added to the cell mixture, the ability of the NK cells to kill Daudi cells is dramatically inhibited and the addition of IL-2 cannot overcome this suppression caused by the monocytes (purple and green bars, right panel). Histamine, however, by blocking the monocyte-induced ROMs, can reverse the monocyte-induced suppression and thereby restore the enhanced NK cell killing induced by IL-2 (orange and blue bars, right panel).

Based on these results, a logical therapeutic strategy would be to develop a method to inhibit the phagocyte-induced suppression of NK cells and T-cells at the site of the tumor. Such inhibition of the phagocyte-derived ROMs could allow for more effective activation and enhancement of NK cells and T-cells by cytokines or other

immunomodulating treatments. Subsequent *in vitro* and *in vivo* research by Hellstrand and coworkers has shown that histamine safely and effectively blocks ROM generation by phagocytic cells and therefore protects and improves NK cell and T-cell activation, and cytotoxic activity.[58,60-64] Histamine appears to have a synergistic effect with IL-2 or IFN-α activation of NK cells and T-cells leading to increased destruction of tumor cells. An example of the ability of histamine to synergize with IL-2 and/or IFN-α for the activation of T-cells is shown in Figure 2.



From: Hansson. Data on file, Maxim Pharmaceuticals 1999.

Figure 2. Enhanced Activation of CD8+ T-cells by Histamine

Figure 2 clearly demonstrates that monocytes significantly reduce the number of activated T-cells in the presence of IL-2 or IFN-α and that the addition of histamine reverses this suppression and significantly increases the percentage of activated T-cells up to 62 fold over that seen with IL-2 alone.[64]

In support of this hypothesis, it has been shown that the presence of intratumoral macrophages in primary melanomas correlates with the subsequent development of metastases.[65] Monocytes and macrophages isolated from melanoma metastases inhibit NK and T-cell activity by secreting ROMs *in vitro*.[66] In addition, a positive correlation has been demonstrated between phagocyte infiltration of primary colorectal carcinomas and the propensity of these tumors to produce distant metastases.[55] The prognostic importance of this mechanism of NK cell inhibition has been demonstrated by comparing the degree of NK cell inhibition at various stages of colorectal tumors. It was found that intratumoral inhibition of NK cells was more

pronounced in Dukes' B and C tumors (advanced stage of disease) than in Dukes' A type tumors which have a more favorable prognosis.[67]

NK and T-cell Activation by Cytokines and Histamine

Understanding the interactions between NK cells, T-cells and phagocytes not only helps to explain the somewhat limited efficacy of IL-2 and IFN-α in cancer patients, but also sets the stage for the therapeutic role of histamine. Given that NK and T-cells are activated by and, thus, should participate in the antitumor efficacy of IL-2 and IFN-α; cytokine therapy should be more efficacious if NK and T-cell activity were not inhibited by phagocyte derived ROMs. A drug that prevents ROM inhibition of lymphocyte activity should enhance cytokine therapy, especially in tumors or tissue high in phagocyte concentration. Hence the role for histamine.

The biogenic amine, histamine, does not stimulate the cytotoxicity of lymphocytes by itself. However, histamine does inhibit the generation of ROMs by phagocytes.[68] By this mechanism, histamine strongly synergizes with IL-2 and IFN-α to activate lymphocyte killing of a variety of cultured tumor cells as well as solid and leukemic cells recovered from cancer patients.[69,70] This effect of histamine is mediated strictly through histamine receptors of the H_2 subtype, which are present on phagocytes. The receptor specificity of histamine's effects on phagocytes is supported by its inhibition by histamine antagonists and reproduction by other H_2 agonists.[71-74]

The effect of histamine can be demonstrated in ^{51}Cr-labeled acute myelocytic leukemia blasts isolated from a patient in blast crisis.[71,72] Labeled blast cells were incubated with a fixed number of enriched NK cells and varying amounts of monocytes for 16 hours. The percentage of blasts killed was estimated by measuring the amount of radioactivity release from blasts with disrupted cell membranes. Interleukin-2 effectively induced NK cells to kill blasts (about 60% cell death). The presence of monocytes almost completely blocked this response, demonstrating that IL-2 was incapable of activating NK cells in the presence of monocytes (presumably due to release ROMs). When histamine was added with IL-2, monocyte inhibition of IL-2 induced activation of NK cells was prevented. Interleukin-2 and histamine synergistically activated NK cells and maximized blast cell death.

Tumor Response Assays Using Histamine

The initial report on the effect of histamine on tumor growth was published by Burtin *et al.*[75] They injected 1 x 10^4 methyl cholanthrene-induced sarcoma cells subcutaneously into C57BL/6 mice. One day later, the mice were randomly assigned to receive daily injections of either saline, 1.8 mg of histamine dihydrochloride, or 6 mg of histamine dihydrochloride. These injections continued for the duration of the study. Tumors became palpable on day 11 after sarcoma cell injection in mice that received saline. In contrast, the tumor did not become palpable until day 13 in both of the histamine treated groups. In the same report, Burtin *et al.,* reported on injecting subcutaneously a < 1 mm^3 piece of the same strain of tumor into C3H mice. These mice were also divided into three treatment groups that received daily injections of either saline, 1.8 mg of histamine, or 6 mg of histamine. In this trial however, the histamine therapy did not begin until 5 days after tumor inoculation. Half of the animals in both of the histamine treated groups were classified as responders, although the definition of what constituted a responder appears to have been made post hoc.

There were no further reports in this area of investigation, until the work of Hellstrand *et al.,* began in the mid 1980s. Hellstrand's group conducted studies similar to those of Burtin *et al.*, but in a more definitive and structured manner. They were also the first to identify the immunologic basis for the histamine effect.

The initial report of the immunologic effect of histamine on tumor growth was made by Hellstrand *et al.*[76] Swiss albino mice were inoculated intravenously with either B16 F1 or B16 F10 melanoma cells. One day prior to the tumor cell injection, the mice were administered either histamine, the H_2 antagonist ranitidine, the H_2 agonist dimaprit, or a control solution. Mice were sacrificed 14 to 21 days after tumor injection and the number of pulmonary metastatic foci (PMF) and the tumor mass (TM) were measured. The individual experiments were performed with 5 to 10 animals in each treatment group.

The results of this study are summarized in Table 1, with the data expressed as percent of control value. Histamine decreased the number of PMF as well as TM. This effect was dose dependent - a greater effect was seen with 250 mg/kg than with 25 mg/kg histamine. Interestingly, the H_2 receptor antagonist ranitidine resulted in a several fold increase in the number of PMF and TM in a dose dependent manner.

Table 2. Effects of Histamine, Ranitidine, Dimaprit, and *nor*-Dimaprit on B16 Melanoma Metastasis

					% of Control (mean ± SEM)	
Exp. No.	**Tumor Cell**	**Treatment**	**Dose (mg/kg)**	**N**	**PMF**	**TM**
1-9	B16 F1	Control	--	78	100 ± 7	100 ± 9
		Histamine	250	27	16 ± 3[a]	13 ± 9[a]
		Histamine	25	32	38 ± 4[a]	38 ± 7[a]
		Ranitidine	50	40	404 ± 93[a]	315 ± 98[a]
10-16	B16 F10	Control	--	31	100 ± 8	100 ± 9
		Histamine	250	15	18 ± 9[a]	24 ± 10[a]
		Histamine	25	20	54 ± 7[a]	44 ± 8[a]
		Ranitidine	50	27	235 ± 60[a]	215 ± 61[a]
17	B16 F1	Control	--	10	100 ± 11	100 ± 5
		Histamine	250	5	11 ± 5[a]	5 ± 1[a]
		Histamine	25	5	27 ± 5[a]	11 ± 1[a]
		Histamine	2.5	5	56 ± 6[a]	29 ± 3[a]
		Dimaprit	250	5	10 ± 3[a]	10 ± 5[a]
		Dimaprit	25	5	55 ± 14[a]	39 ± 10[a]
		Dimaprit	2.5	5	103 ± 11	94 ± 4
18	B16 F10	Control	--	20	100 ± 5	100 ± 13
		Ranitidine	25	10	208 ± 24[a]	271 ± 28[a]
		Histamine	10	10	58 ± 5[a]	65 ± 10[a]
		Histamine + Ranitidine	2.5	10	216 ± 24	267 ± 38
		Dimaprit	10	10	74 ± 5[a]	60 ± 14[a]
		Histamine + Ranitidine	25	10	217 ± 34	263 ± 34
		Nor–Dimaprit	10	10	100 ± 5[b]	97 ± 9[a]

a) $p < 0.01$ versus control (analysis of variance followed by Fisher's protected least significant difference test (two-sided) on logarithmic values).
b) $p < 0.01$ versus control, $p < 0.02$ versus dimaprit.

Histamine administration decreased both the number of tumor metastases and the total mass of the metastases in a dose dependent manner, as did the H_2 agonist dimaprit. The H_2 receptor antagonist had the opposite effect. These findings support the hypothesis that histamine can prevent NK cell suppression by phagocyte-generated ROMs, that this effect is mediated through the H_2 receptor, and thus histamine is either preventing the development and/or growth of tumor metastases.

In the same manuscript, Hellstrand *et al.* repeated these same tumor response assays but added IL-2 at a dosage of 6000 U/kg as one of the treatment parameters. The results from these studies are summarized in Table 2. As can be seen, results similar to those obtained in the earlier studies were observed when using histamine and ranitidine alone. In addition, IL-2 was found to be efficacious in decreasing both PMF numbers and TM. More importantly, the combination of IL-2 and histamine was more efficacious than were either of these agents alone, and this effect was blocked by the histamine antagonist ranitidine.

Table 3. Effects of Histamine, Ranitidine, and IL-2 on B16 Melanoma Metastasis *In Vivo*

Exp. No.[a]	Mouse/B16 Strain	Tumor Parameter	Lung Tumors after Treatment[a] with:					
			Control	Histamine	Ranitidine	IL-2	Histamine + IL-2	Ranitidine + IL-2
1[b]	Swiss/F1	PMF	159 ± 43	41 ± 15	363 ± 63	44 ± 11	0	350 ± 56
		TM	116 ± 36	37 ± 21	231 ± 63	29 ± 24	0 ± 0.3	257 ± 14
2[b]	Swiss/F1	PMF	32 ± 7	14 ± 2	319 ± 79	8 ± 3	0	375 ± 78
		TM	15 ± 4	7 ± 1	295 ± 85	7 ± 4	0 ± 0.4	327 ± 85
3[b]	Swiss/F1	PMF	49 ± 9	19 ± 3	ND†	16 ± 2	0	ND[c]
		TM	93 ± 10	74 ± 19	ND†	38 ± 12	0 ± 10	ND[c]
4[b]	Swiss/F10	PMF	547 ± 71	232 ± 61	>700	255 ± 49	38 ± 10	ND[c]
		TM	261 ± 31	89 ± 39	477 ± 69	92 ± 10	9 ± 3	ND[c]
5[b]	C57B/F10	PMF	171 ± 20	73 ± 14	>500	84 ± 17	3 ± 2	ND[c]
		TM	131 ± 15	53 ± 16	497 ± 79	79 ± 8	1 ± 0.5	ND[c]

a) All compounds were administered I.V. as a single dose 24h before I.V. inoculation of 10^5 B16 melanoma cells (histamine 25mg/kg; ranitidine 50mg/kg; IL-2 6000 U/kg). The results shown were obtained in 5 separate experiments.

b) Statistical evaluation (PMF values, Mann-Whitney *U*–test): Exp. 1-3: control vs. histamine, IL-2, ranitidine, histamine + IL-2, or ranitidine + IL-2: $p < 0.01$; histamine + Il-2 vs. histamine or IL-2: $p < 0.01$; ranitidine + IL-2 vs. IL-2: $p < 0.01$; ranitidine vs. ranitidine + IL-2: $p > 0.1$.
Exp. 4: control vs. histamine or IL-2: $p < 0.05$; histamine + IL-2 vs. histamine or IL-2: $p < 0.01$.
Exp. 5:control vs. histamine, IL-2, or histamine + IL-2: $p < 0.01$; control vs. ranitidine: $p < 0.05$; histamine + IL-2

c) ND, not done.

In summary, these data suggest that there is a potential synergy between IL-2 and histamine to reduce PMF and TM when used as a combination immune therapy in this model. This antitumor effect was mediated by H_2 receptors and was reversed by histamine H2 receptor antagonists.

There have been two other recent reports[77,78] conducted by other investigators that demonstrated a protective effect of histamine on host response to tumor growth. Rovere *et al.*[77] injected 1 x 10^3 Yoshida ascite sarcoma cells intraperitoneally. The animals received either saline or 0.005 µg of histamine per rat for varying periods in relation to the time of tumor challenge. These time periods included: 1) days –3 to –1; 2) days –15 to –1; 3) days –15 to +20; and 4) days +1 to +20. For this study, long term survival was deemed to have occurred if an animal survived to > 60 days. The long term survival rates for this study were 0% for saline treated animals, 0% for the animals that were administered histamine from days –3 to –1, 30% for the animals that were administered histamine from days +1 to +20, 70% for the animals that received histamine from days –15 to –1, and 80% for the animals that received histamine from days –15 to +20. The survival rates for the latter two groups were statistically significantly greater than the saline control groups. These findings suggest that histamine can be efficacious in certain oncologic situations, especially during the early stages of the oncologic process, i.e., when NK cells are believed to be pivotal in protecting the host.

Suonio *et al.*[78] reported on taking colonic tumor cells from ten patients with colon cancer. The tumor cells were injected into the subrenal capsule of mice. The mice were administered daily injections of a number of drugs including 1 mg/kg of histamine and 100 mg/kg of the H_2 antagonist cimetidine. Six days later the animals were sacrificed and the tumor size was measured. It was found that histamine had decreased tumor growth compared to the saline control group.

In Vivo Cytotoxicity Assays with Histamine

The effect of histamine on natural (NK) cell function was also evaluated by Hellstrand using the *in vivo* mouse model assay developed by Hanna and Fidler.[79] In this model, mice were injected with histamine, ranitidine, or a control solution. Twenty-four hours later they were injected intravenously with ^{51}Cr-labeled YAC-1 lymphoma cells, which are extremely sensitive to lysis by NK cells. Four hours after the YAC-1 injection the animals were sacrificed and the amount of radioactivity in their lungs was assayed. The radioactivity in lungs has been shown to be inversely proportional to NK cell function (i.e., the less radioactivity in the lungs, the greater the

lysis by NK cells of the target YAC-1 cells which form tumor emboli in the lungs).

The results of this study are summarized in Table 3. Histamine treatment decreased the amount of lung radioactivity by two thirds compared to the control group, whereas the H_2 antagonist ranitidine tripled the amount of radioactivity. This suggests that histamine significantly increased the effectiveness of NK cells in killing YAC-1 cell tumor emboli by preventing NK cell suppression by ROMs. The inhibition of this effect by blockade of H_2 receptors decreased NK cell lysis of YAC-1 cells by two thirds indicating that the effect is mediated through H_2 receptors. Co-administration of histamine and the H_2 antagonist ranitidine produced a similar result, as did ranitidine therapy alone.

Table 4. Effects of Histamine and Ranitidine on Clearance of YAC-1 Lymphoma Cells in Lungs

Exp. No.*	Retained Radioactivity after Treatment[a] with:		
	Ranitidine	Vehicle	Histamine
1	-	20.5 ± 2.5	6.0 ± 0.5[b]
	+	56.4 ± 4.3[b]	61.3 ± 1.8[c]
2	-	9.6 ± 1.0	3.1 ± 0.9[b]
	+	22.1 ± 2.9[b]	19.5 ± 1.7[c]

a) Treatment was administered I.V. as a single dose 24h before I.V. inoculation of 10^5 ^{51}Cr-labeled YAC-1 cells. Histamine was used at 125 mg/kg, ranitidine at 50 mg/kg. The results show retained radioactivity (mean of 4 animals ± SEM) in lung tissue (percent of radioactivity retained in lungs at time zero after injection of labeled tumor cells) of Swiss albino mice.
b) $p < 0.01$ versus control (Mann-Whitney *U*-test).
c) $p < 0.01$ versus control, $p > 0.1$ versus ranitidine.

Asea *et al.*[63] conducted a similar series of studies to those of Hellstrand however, they utilized not only the NK cell sensitive B16 melanoma cells and YAC-1 lymphoma cells, but also tested histamine against NK cell resistant P815 mastocytoma cells. In addition, in some groups they administered either anti-NK1.1 or anti-asialo-GM1 antibodies, both of which are known to deplete NK cells. They tested the effects of IL-2 and IFN-α as an immune enhancing cytokine in this model.

Results of the study are summarized in Tables 4 and 5. As in the earlier study by Hellstrand, ranitidine administration resulted in an increase in the amount of radioactivity remaining in the lungs four hours after injection of radiolabeled YAC-1 tumor cells, whereas histamine decreased this amount. Neither histamine nor ranitidine were reported to have any activity when the tumor cell utilized was the NK cell-resistant P815.

Interferon-alpha was found both to decrease slightly the number of metastatic foci following B16 melanoma cell injection and the number of remaining tumor cells following YAC-1 lymphoma cell injection. A similar decrease was seen with histamine therapy. The most significant effect was seen when histamine was combined with INF-α, which produced a large reduction in metastatic foci and remaining tumor cells (Table 5).

Table 5. Time-Dependent Effects of Histamine and Ranitidine on NK Cell Cytotoxicity *In Vivo*

(A)	Remaining radioactivity In lungs (%) after treatment with:		
Treatment	Control	Histamine	Ranitidine
Control	24.0 ± 1.0	12.1 ± 1.1	34.5 ± 3.0
Anti-NK1.1	44.0 ± 3.1	45.9 ± 4.0	49.4 ± 3.9
(B)	Remaining tumor cells in lung (% of control) after treatment for:		
Treatment	Dose (mg / kg)	5 min	180 min
Control	--	100 ± 11	--
Histamine	125	111 ± 17	32 ± 9[a]
(C)	Remaining radioactivity (cpm) in lungs after treatment with:		
Time after TCI	Control	Histamine	Ranitidine
T = 1 min	30,184 ± 160	32,212 ± 837	31,102 ± 430
t = 2 h	3,254 ± 780	1,551 ± 169[b]	10,688 ± 1,939[b]

(A) Animals were depleted of NK cells with anti-NK1.1 inoculated I.V. 24 h before treatment with histamine or ranitidine, which in turn was given 180 min before the I.V. inoculation of 100,000 radiolabeled YAC-1 cells. Data are retained radioactivity in lungs at 120 min after tumor cell inoculation (TCI), expressed as the percentage of the radioactivity retained in untreated animals at t = 1 min after injection of tumor cells to untreated animals.

(B) Histamine (125 mg/kg) was injected I.V. 5 or 180 min before I.V. inoculation of 100,000 radiolabeled YAC –1 cells. Each value (percentage of control animals with 9.6 ± 1.0 % remaining tumor cells) is the mean ± SEM of 4 – 8 animals of the radioactivity retained in lungs 2 h after TCI.

(C) Histamine (125 mg/kg) or ranitidine (50 mg/kg) were injected 3 h before inoculation of 100,000 radiolabeled YAC-1 cells. Animals were killed at 1 or 120 min after TCI. Data are cpm in lungs ± SEM of 4 – 5 animals.

a) $2p < 0.005$ versus control, $2p < 0.007$ versus histamine t = 1 min (Student's t-test).

b) $2p < 0.02$ versus respective controls.

Table 6. Effects of Histamine and IFN-a on B16 Lung Metastasis and Clearance of YAC-1 Lymphoma Cells in Mice *In Vivo*

(A)	Metastatic foci[a]	
Treatment[b]	**Medium**	**IFN-a**
Medium (control)	33.9 ± 8.4	8.6 ± 2.0
Histamine	25.5 ± 7.0	1.0 ± 0.6[c]
(B)	**Remaining tumor cells (%)[a]**	
Treatment[b]	**Medium**	**IFN-a**
Medium (control)	52.4 ± 5.1	42.9 ± 1.9
Histamine	22.1 ± 2.8	11.9 ± 0.9[d]

a) All compounds were injected I.V. 6 h before inoculation of (A) 100,000 B15/F10 melanoma cells or (B) of 100,000 radiolabeled YAC-1 cells. Each value is the mean ± SEM of 4 – 5 animals of the number of tumors visible on the lung surface at 3 weeks (A) after tumor cell inoculation or (B) the radioactivity retained in lungs 1 – 2 h after inoculation of tumor cells, expressed as the percentage of radioactivity retained at t = 1 min after injection of tumor cells. Neither of the compounds used affected the radioactivity retained in lungs at t = 1 min after injection of labeled tumor cells (not shown).

b) Histamine: 50 mg/kg; IFN-α: 0.5×10^4 U/μg.

c) $p < 0.04$ versus histamine, $2p < 0.006$ versus IFN-α (Student's t-test)

d) $2p < 0.01$ versus histamine, $2p < 0.01$ versus IFN-α (Student's t-test)

In summary, multiple animal models have shown that histamine administration improved *in vivo* NK cell clearance of YAC-1 tumor cells. The use of an H_2 receptor antagonist impaired NK cell function, and the combined use of both histamine and its antagonist had a similar effect as the antagonist alone. Histamine plus IL-2 and /or IFN-α showed synergistic antitumor efficacy. These data suggest that histamine improves NK cell function by both enhancing its activation by IL-2 and preventing ROM inhibition of NK cell activation.

1.3.1 Metabolism

Histamine is rapidly degraded by a wide range of tissues *in vivo*.[97] There are two major paths of histamine metabolism in humans.[98] The more important of these involves ring methylation and is catalyzed by histamine-N-methyltransferase, an enzyme widely distributed in the body. Most of the product, N-methylhistamine, is converted by monoamine oxidase (MAO) to

N-methyl imidazole acetic acid. This reaction can be blocked by MAO inhibitors.[98,99] Alternatively, histamine undergoes oxidative deamination catalyzed by diamine oxidase (DAO), also known as histaminase. Diamine oxidase is found at high levels in the intestinal epithelial cells, kidney, thymus, decidual placenta, and in low levels elsewhere.[98] Diamine oxidase converts histamine to imidazole acetic acid, which in turn may be conjugated with ribose by the enzyme imidazole acetate ribosyltransferase. The principal sources of the ribosyltransferase in the rat are liver and kidney.[98]

The major metabolites found in the urine of healthy persons are N-methylhistamine and N-methyl imidazole acetic acid.[100] The normal daily urinary excretion of histamine, N-methylhistamine, and N-methylimidazole acetic acid is 0.023, 0.19, and 2.7 mg respectively.[98]

There is no evidence that histamine is metabolized via the usual drug-metabolizing cytochrome P_{450} systems. However hepatocytes have cytochrome P_{450} enzymes as a major component of microsomal intracellular sites and it has been demonstrated *in vitro* that polyamines compete with high affinity with histamine for these binding sites.[101]

In man, the radioactivity of exogenously-administered ^{14}C-histamine is largely excreted in the urine during the first 6 hours.[99] There is no evidence of significant storage of ^{14}C-histamine or of metabolism which splits the imidazole ring.

1.3.1.1 Activity and Pharmacokinetics of Metabolites

Under physiological conditions, the metabolites of histamine have very little or no activity and are excreted in the urine.[102] However imidazole acetic acid (IAA) can show analgesic and narcotic action at doses above 50 mg/kg *in vitro*, and is chemoattractant for eosinophils.[98] Also *in vitro*, IAA inhibits histaminase release from polymorphonuclear leukocytes (PMN), and is implicated in a highly specific effect in complement-mediated PMN function.[103]

The metabolite, methyl-histamine, has a similar biological half-life to histamine in the rat.[104] The renal extraction ratio of histamine from human kidneys is 0.7-0.8. This high extraction ratio for whole blood implies that a tubular transport mechanism in the kidney is responsible for renal removal of histamine from the blood. There appears to be a continuous metabolism of histamine and methylhistamine.[105]

2. Phase 2 Studies

Two early phase 2 studies, evaluating the safety and efficacy of subcutaneous injections of histamine in patients receiving IL-2 and IFN-á as treatment for metastatic melanoma, were conducted in Sweden. Reports were made available to Maxim and they were designated MM-1 and MM-2.

2.1 Study MM-1

The objectives of study MM1 were to investigate the safety and potential efficacy of histamine dihydrochloride as an adjunct to IL-2 and IFN-α in patients with stage IV melanoma. A total of 17 patients were enrolled from August 15, 1989 to May 5, 1993. Survival data on all patients was tabulated as of November 12, 1999.

Study MM-1 was an open-label, controlled, non-randomized sequential study conducted by principal investigators Peter Naredi, M.D., Ph.D. and Per Lindnér, M.D., Ph.D. at the Sahlgrenska University Hospital in Gothenburg, Sweden in treatment naive patients with advanced stage IV melanoma.[95] Seven patients were enrolled and given intermediate dose continuous intravenous infusions of IL-2 (18 x 10^6 IU/m^2 per day) plus SC IFN-α (3 x 10^6 IU/m^2 per day). The cytokines were given five times per week for two weeks, followed by a three-week intermission, after which most of the patients were recycled. Patients with stable disease or a response after two cycles were administered additional one-week courses of therapy every fourth week until disease progression was noted. The subsequent ten patients received the same dose and dosing schedule of IL-2 and IFN-α but were also given histamine dihydrochloride at a dose of 1 mg by subcutaneous injection on a twice daily basis during the IL-2 dosing days.

Tumor size was estimated at baseline and thereafter approximately every eighth week. Assessment was performed by x-ray, CT scan, MRI scan, ultrasound, and physical palpation. The overall response involved an assessment of both measurable and non-measurable disease and a calculation by the investigator of the patient's total tumor burden as compared to baseline. Duration of survival was determined from the time of first dose until the time of death.

Each site of tumor was evaluated as a complete response (CR); a partial response (PR), stable disease (SD) or progressive disease (PD) based on tumor volume. CR was defined as complete resolution of all detectable tumor mass. PR was defined as more than 50% regression of tumor volume. SD was defined as anything less than PR without PD.

Adverse events, signs and symptoms, and hematology and serum values were monitored throughout the study.

One patient did not meet the inclusion criteria (Karnofsky Performance Score < 70) but received histamine dihydrochloride on a "named patient license", a regulatory aspect of Swedish law allowing treatment use of an investigational drug. The patient was excluded from the efficacy analysis of the study but was included in the safety analysis only. The efficacy evaluable population in this study consisted of Treatment Group A (control group) with seven patients and Treatment Group B (histamine group) with nine patients.

Efficacy Assessment

The treatment groups in this study were well matched for prognostic factors such as age and number of metastatic sites. Five of 16 patients had liver metastases at inclusion (3 in treatment group A and 2 in treatment group B), 12 had visceral metastases (5 in group A and 7 in group B) and the mean number of metastatic sites was 3.0 (2.6 in group A and 3.3 in group B). These baseline data indicate poor prognostic factors for limited duration of expected survival.

The median duration of survival was 7.6 months in group A and 16.3 months in group B. The mean duration of survival (± SD) was 8.5 ± 6.0 for group A and 18.0 ± 15.7 for group B. There was one partial responder in group A (14%) whereas in group B there were 3 partial responders (33%) and 2 patients (22%) with stable disease as best overall response. None of the 3 patients with liver metastases in group A responded while both patients (100%) with liver metastases in group B had an overall partial response and both had a complete response of their liver metastases. The survival results are summarized in Table 7.

Table 7. Duration of Survival in Study MM-1

	Duration of Survival (months)[a]	
	Group A (N = 7) (Control)	**Group B (N = 9) (Histamine Treated)**
Median	7.6	16.3
Mean (± SD)	8.5 (± 6.0)	18.0 (± 15.7)
Min – Max	0.6 – 15.3	3.2 – 56.3
95% CI Lower Limit	0.6	6.2
95% CI Upper Limit	15.4	23.5

[a] Time from First Dose of Study Drugs to Death by any Cause

Because this study used a sequential assignment of patients to treatment groups, the improvement in response and duration of survival in the patients who received histamine, could be attributed to factors other than the histamine. Nevertheless, the results of this study are positive and suggest that histamine when used in combination with IL-2 and IFN-á may be effective.

Safety Assessment

The extent of exposure to study drugs was greater for patients in group B (who received histamine) than for patients in group A (95 vs. 64 days). There was no difference in the ratio of actual administered dose vs. intended dose for IL-2 and IFN between the two groups.

Adverse events reported in this study were generally mild or moderate. Adverse events related to IL-2 and IFN treatment were essentially similar to those described in the literature for these cytokines. Fever, chills, asthenia, rash, diarrhea and vomiting were most frequently reported. There was no clinically significant difference in these adverse events reported between the two groups. In group B (receiving histamine), headache and hypotension were reported more often. This is believed to be due to the short-lasting vasodilatation associated with administration of histamine dihydrochloride. Three of 10 patients had dose reductions of histamine dihydrochloride.

There were no treatment-related deaths during the study and all patients except one died from melanoma. Patient #4 in group A died from a pulmonary embolus 3 months after treatment was completed. Of the 8 Serious Adverse Events (SAE) recorded in 6 patients (4 in group A and 2 in group B) none resulted in death. Three were related to metastases of melanoma and the others were respiratory insufficiency (group A), infection (group A), cardiac arrhythmia (group A) and sepsis with pleural effusion (group B). Changes in laboratory parameters were similar in both populations, were trendless, and were rarely clinically significant.

Due to the toxicity of IL-2 at the doses tested and to further assess the role of histamine, a new protocol was developed to allow for out-patient subcutaneous administration of a lower dose of IL-2. The second study was designated as MM-2.

2.2 Study MM-2

The objective of study MM-2 was to determine if the addition of histamine to IFN-α and a lower dose of IL-2 would be safe and potentially efficacious in patients with advanced stage IV melanoma. The study population was divided into 2 groups, designated group A and group B. Patients in group B received a higher dose of IL-2 as an initial bolus continuing then with the lower dose for the remainder of the cycle and daily administration of histamine throughout the treatment period. This protocol was a prospective, single arm trial in which all patients received histamine in addition

to the cytokines. There was no control group. The evaluable population consisted of 14 patients in group A and 13 patients in group B. A total of 32 patients received histamine injections and thus constitute the safety population.

In this trial the dose of IFN-α was the same as in the MM-1 study (3×10^6 IU/day) by subcutaneous injection. The IFN-á injections began 7 days prior to the histamine and IL-2 injections and continued daily throughout the treatment cycle (days - 7 to 28). Histamine dihydrochloride was given as a 1 mg subcutaneous injection twice daily for two, 5-day periods (Days 1-5 and 8-12) separated by 2 days of rest. In group A, IL-2 was given on the same days as histamine, also twice per day as a subcutaneous injection of 2.4×10^6 IU/m^2. This treatment was followed by a 2-week intermission from IL-2 and histamine therapy, before recycling the patients. This cycling sequence was repeated until disease progression occurred.

Patients in group B received the same dose of IFN-á (3×10^6 IU/day) by subcutaneous injection daily throughout the treatment cycle (days - 7 to 28). The treatment regimen for patients in group B differed from that for group A in that bolus doses of IL-2 (10×10^6 IU/m^2) were administered twice daily on days 1 and 2 of each treatment cycle with doses of 2.4×10^6 IU/m^2 given on days 3-5 and 8-12. Histamine dihydrochloride was given BID on every day of the treatment cycle, including the days when the IFN-á priming doses were given.

The patient population and the protocol-specified assessments of efficacy and safety were the same in this study as they were in Study No. MM-1 except that most patients self-administered IL-2/IFN-α and histamine on an out-patient basis. Thirty-two (32) patients were enrolled in study MM-2 beginning in July 1994. The last patient completed treatment in September 1999. Five additional patients were treated with the same protocol on individual named patient licenses and their data is included in the safety analysis only. Survival data on all patients was tabulated as of November 12, 1999.

Efficacy Assessment

The median duration of survival was 15.1 months in population A and 8.0 months in Population B. The survival results are summarized in Table 8.

Table 8. Duration of Survival in Study MM-2

	Duration of Survival (months)[a]	
	Population A (N = 14)	**Population B (n = 13)**
Median	15.1	8.0
Mean (± SD)	17.5 (± 10.7)	11.0 (± 8.0)
Min – Max	5.9 – 44.6	2.5 – 31.3
95% CI Lower Limit	6.5	4.8
95% CI Upper Limit	27.0	18.6

[a] Time from First Dose of Study Drugs to Death by any Cause

The duration of survival is longer than would be expected based on previous reports and the status and tumor burden of the patients upon entry into the study and further suggests the possible efficacy of histamine used adjunctively with cytokine therapy in advanced malignant melanoma.

Safety Assessment

The study population was comprised of severely ill patients and adverse were reported in all 32 patients. The addition of histamine dihydrochloride is probably responsible for the occurrence of some additional adverse events. Vasodilation, fever, chills, asthenia, nausea, headache, taste perversion, and anorexia were the most frequently reported adverse events.

There were no treatment-related deaths during the study. Thirty-one of 32 (96.9%) patients had died from melanoma at the time of the data cut-off on November 12, 1999. Ten patients experienced a total of 12 serious adverse events.

2.3 Analysis of Patients with Liver Metastases in Studies MM-1 and MM-2

Liver metastasis is known to be a negative prognostic indicator for survival in stage IV melanoma and thus it was important to examine the survival of these patients in studies MM-1 and MM-2.

Of the 49 patients enrolled in studies MM-1 and MM-2, 15 (31%) had liver metastases. An analysis of survival for the patients with liver metastases, drawn from both studies, was performed. The patients with liver metastases were mostly male (10 male and 5 female with a mean age 51.6 years)

The survival results for the liver metastasis patients combined from studies MM-1 and MM-2 are summarized in Table 9.

Table 9. Duration of Survival (Days) for Liver Metastasis Patients in Studies MM-1 and MM-2

	Survival Time (Months)
Median	10.8
95% CI (Lower Bound)	6.2
95% CI (Upper Bound)	14.0
Mean (± SD)	14.6 (± 11.0)
Min – Max	2.5 – 44.6

The duration of survival for patients with liver metastases in studies MM-1 and MM-2 was notably longer than survival times reported in the medical literature (4-5 months) for such patients and led to the inclusion of patients with liver metastases at baseline as a primary analysis population to be evaluated in the randomized phase 3 trial.

3. Phase 3 Trial

3.1 Study Methods

3.1.1 Study Design

Maxim clinical study MP-US-M01 (also referred to as M01) was a multicenter, phase 3, randomized, controlled, parallel-group, open-label clinical trial evaluating the effect of a combination treatment on the duration of survival of patients with metastatic melanoma. Patients were randomized to receive IL-2 plus or minus histamine by subcutaneous injection. The study was conducted at 56 institutions in the United States. Enrollment was closed on March 8, 1999[a]. Follow-up continued through March 8, 2000, at which time 300 patients (the original planned sample size) would each have reached a minimum of 12 months follow-up.

[a] On March 8, 1999 when the 300th patients was randomized there were five additional patients in screening who were allowed to enroll, thus the final enrollment was 305 patients. The last patient was randomized on March 26, 1999.

3.1.2 Study Management

Study M01 was conducted under IND 52,603, originally filed in January 1997. Monitoring and data management were not done by sponsor, but were originally transferred to Covance Clinical and Periapproval Services of Nashville, TN. This function was later transferred to IBAH Clinical Research (now Omnicare Clinical Research). Qualified personnel from Maxim Pharmaceuticals also performed some site monitoring and audit functions. The randomization of patients to one group or the other was accomplished by giving instructions to investigators by telephone; the randomization was administered by Covance Clinical and Periapproval Services, Nashville, TN. After determining eligibility for enrollment for a patient, principal investigators telephoned Covance to determine assignment to treatment arm. Medical monitoring was also performed by Covance as well as safety reporting. Maxim was contacted regarding protocol exceptions during the study.

A Drug Safety Monitoring Board (DSMB) was utilized in study M01 and all other ongoing Phase 3 studies. Safety data were reported monthly to the DSMB by Covance. On one occasion the DSMB also reviewed interim efficacy data in order to assess futility of continuing the study. The study was allowed to continue after the DSMB closed review.

Survival results and all other efficacy data were managed by Covance and later Omnicare. Efficacy results were embargoed from release to the sponsor until April 11, 2000 at which time the first tabulated results were reviewed.

3.1.3 Objectives

The primary objective of study M01 was to assess the safety and efficacy of the combination of histamine dihydrochloride and subcutaneous IL-2 on the duration of survival of patients with metastatic melanoma. Secondary objectives included assessment of time to disease progression, tumor response, quality of life, and safety.

3.1.4 Entry Criteria

Patients who satisfied the following criteria were enrolled in the study. Exceptions to these criteria were discussed in advance with the medical monitor of the study and the sponsor before allowing such patients to participate.

3.1.3.1 Inclusion Criteria

1. Males and females 18 years and older with melanoma which had progressed to Stage IV malignant melanoma.

2. Patients may have been untreated, or may have received previous regimens of chemotherapy, radiation therapy, immunotherapy other than with IL-2, and/or surgery.

3. There must have been one or more bidimensionally measurable masses (in some cases, unidimensional lesions were acceptable), evaluated using the metric system, taken within 3 weeks of the on-study date. Measurements may have been made by x-ray, computed tomography scans (CT), or magnetic resonance imaging (MRI) scans, palpation, or other acceptable methods of measurement. Skin lesions must have been documented by medical photography. Initial brain scans should have been with MRI. Lesions such as skin nodules or superficial lymph nodes that could be evaluated by clinical examination or tumors with clear circumferences on x-ray, CT, or MRI scans were considered measurable. Measurable lesions must have been of the following minimum size: for a single lesion 20 x 20 mm or greater bidimensionally; for patients with multiple lesions the minimum size was 10 x 10 mm for at least one lesion and unidimensional lesions were also acceptable if greater than 20 mm in any direction. Nuclear medicine bone scans, pulmonary lymphangietic metastases, and blastic bone lesions on skeletal x-ray were not considered measurable or evaluable. Ascites and pleural effusions were not considered as measurable but were evaluable.

4. Patients with prior radiation therapy were to be allowed, provided that the indicator lesion(s) was (were) outside the field of radiation or represented new lesions appearing in the radiation field.

5. Patients with prior radiation therapy to the indicator lesion were eligible if the radiation therapy occurred greater than 30 days prior to randomization.

6. A palpable mass or diffuse hepatomegaly that could not be measured, abnormal serological tests of liver function, or serological tumor markers were not evaluable.

7. Life expectancy of 3 months or more and were able to undergo routine outpatient evaluations for efficacy, safety, and/or compliance.

8. Clinically adequate bone marrow, kidney, cardiac, and liver function.

9. Hemoglobin greater than 10.0 g/dL, white blood cell count greater than 2,500/mm^3, absolute granulocyte count greater than 1,500/mm^3, platelet count greater than 100,000/mm^3, partial thromboplastin time (PTT) and prothrombin time (PT) within normal limits.

10. Serum creatinine less than or equal to 1.5 mg/dL.

11. Normal cardiac function. For all patients 50 years and older, and patients younger 49 years with a positive cardiac history or an abnormal 12-lead electrocardiogram (ECG) with results noted as abnormal and clinically significant, a cardiovascular stress test was required documenting normal ejection fraction and unimpaired wall motion.

12. Serum bilirubin and aspartate aminotransferase (AST) should have been within normal limits, except for patients with liver involvement for whom serum bilirubin less than 1.5 times the upper limits of normal and AST less than 3 times the upper limits of normal were acceptable.

13. Fasting serum glucose should have been less than 160 mg/dL. Patients with hyperglycemia treated with glyburide were excluded.

14. Patient had recovered from the toxicity of and were expected to receive no other systemic antimalignancy therapy during the study including corticosteroid medication (except as per Section 7 of the protocol) or any investigational drugs within 14 days before initiation of therapy.

15. Women of child-bearing potential must have been non-nursing, non-pregnant and have had a negative pregnancy test within 3 weeks of starting study drug, and must have practiced barrier or oral contraception for the duration of the study, or must have been documented as surgically sterile or one year post-menopausal.

16. World Health Organization (WHO) Performance Status of 0 to 1, which corresponds to a Karnofsky status of 70 or greater.

17. Patient must have been informed of the investigational nature of this study and informed consent obtained.

3.1.3.2 Exclusion Criteria

1. Prior immunotherapy with IL-2.

2. Clinically significant infection defined as any acute viral, bacterial, or fungal infection that required specific therapy. Anti-infectious therapy must have been completed within 14 days of starting study treatment (except for infections acquired during therapy as per Section 7 of the protocol).

3. Abnormal cardiac function.

4. Any concurrent systemic antimalignancy therapy or radiation therapy to measurable malignant masses, except for radiation as palliation treatment for lesions that were not used to measure response.

5. Patient who required steroidal therapy for any reason, anti-hypertensive medications, H_2 (histamine type-2 receptor, H_2R) antagonists (Zantac®, Tagamet®, etc.), or beta-blockers 24 hours prior to first dose of study drug and throughout the duration of the study.

6. No other active malignancies except *in situ* carcinoma of the cervix, localized squamous or basal cell carcinomas of the skin.

7. Primary or metastatic central nervous system malignancy at on-study date, with the exception of ocular melanoma. However, patients with metastasis to the brain that had been completely resected or resolved and controlled could be included in the study provided that approval from the Medical Monitor was obtained prior to randomization. Patients who had received Gamma Knife Radiation must have obtained brain MRI after the procedure was completed, and may have been allowed to enter the pre-study period within 2 weeks of the procedure. Patients who had received any other treatment of brain metastases must have obtained a repeat MRI four weeks post-procedure prior to possible entry into the pre-study period. Patients receiving whole brain radiation were not eligible.

8. Serious recent non-malignant medical complications that, in the opinion of the investigator, made the patient unsuitable for study participation.

9. Organ grafts, with the exception of autologous skin grafts or high-dose chemotherapy with bone marrow or stem cell transplantation.

10. Previous documented history of asthma actively treated in the last 5 years.

11. History of seizures, central nervous system disorders, or psychiatric disability thought to be clinically significant in the opinion of the investigator and adversely affecting compliance to protocol.

12. Medical, sociological or psychological impediment to probable compliance with protocol.

13. Unable to undergo repeat treatments, clinical evaluations, and other diagnostic procedures required by the protocol.

14. Pregnancy or breast-feeding.

15. Hypercalcemia (serum calcium greater than 11.5 mg/dL).

16. Respiratory insufficiency defined as SaO_2 (arterial oxygen percent saturation) < 90% measured by pulse oximetry. If SaO_2 < 90%, a pulmonary function test must have been done, with respiratory insufficiency defined as FEV_1/FVC (forced expiratory volume in one second/forced vital capacity) ratio < 70% of predicted by pulmonary function test.

17. Serum human immunodeficiency virus (HIV) positive or prior history of autoimmune disease (including but not limited to systemic lupus, inflammatory bowel disease, and psoriasis).

18. Receipt of alternative therapies such as laetrile, Brudzinski's treatment, etc.

19. Active peptic and/or esophageal ulcer disease or with past peptic ulcer disease with a history of bleeding.

3.1.4 Treatment Administration

Treatment was given 5 days per week for 4 consecutive weeks, followed by two weeks rest. This 6-week period comprised one cycle. The protocol specified that patients should receive 8 cycles (48 weeks) or should continue treatment until clinical progression of disease was encountered. The dose of both drugs and the study regimens are described in Tables 10 and 11.

Table 10. Dose and Treatment Regimen for Study MP-US-M01 Treatment Arm A (Histamine + IL-2)

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Day 1	IL-2 (9.0 MIU / m^2), BID Histamine 1 mg, BID	IL-2 (2.0 MIU / m^2), BID Histamine 1 mg, BID	IL-2 (9.0 MIU / m^2), BID Histamine 1 mg, BID	IL-2 (2.0 MIU / m^2), BID Histamine 1 mg, BID	Nothing	Nothing
Day 2	IL-2 (9.0 MIU / m^2), BID Histamine 1 mg, BID	IL-2 (2.0 MIU / m^2), BID Histamine 1 mg, BID	IL-2 (9.0 MIU / m^2), BID Histamine 1 mg, BID	IL-2 (2.0 MIU / m^2), BID Histamine 1 mg, BID	Nothing	Nothing
Day 3	Histamine 1 mg, BID	IL-2 (2.0 MIU / m^2), BID Histamine 1 mg, BID	Histamine 1 mg, BID	IL-2 (2.0 MIU / m^2), BID Histamine 1 mg, BID	Nothing	Nothing
Day 4	Histamine 1 mg, BID	IL-2 (2.0 MIU / m^2), BID Histamine 1 mg, BID	Histamine 1 mg, BID	IL-2 (2.0 MIU / m^2), BID Histamine 1 mg, BID	Nothing	Nothing
Day 5	Histamine 1 mg, BID	IL-2 (2.0 MIU / m^2), BID Histamine 1 mg, BID	Histamine 1 mg, BID	IL-2 (2.0 MIU / m^2), BID Histamine 1 mg, BID	Nothing	Nothing
Day 6	Nothing	Nothing	Nothing	Nothing	Nothing	Nothing
Day 7	Nothing	Nothing	Nothing	Nothing	Nothing	Nothing

Table 11. Dose and Treatment Regimen for Study MP-US-M01 Treatment Arm B (IL-2 Alone)

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Day 1	IL-2 (9.0 MIU / m^2), BID	IL-2 (2.0 MIU / m^2), BID	IL-2 (9.0 MIU / m^2), BID	IL-2 (2.0 MIU / m^2), BID	Nothing	Nothing
Day 2	IL-2 (9.0 MIU / m^2), BID	IL-2 (2.0 MIU / m^2), BID	IL-2 (9.0 MIU / m^2), BID	IL-2 (2.0 MIU / m^2), BID	Nothing	Nothing
Day 3	Nothing	IL-2 (2.0 MIU / m^2), BID	Nothing	IL-2 (2.0 MIU / m^2), BID	Nothing	Nothing
Day 4	Nothing	IL-2 (2.0 MIU / m^2), BID	Nothing	IL-2 (2.0 MIU / m^2), BID	Nothing	Nothing
Day 5	Nothing	IL-2 (2.0 MIU / m^2), BID	Nothing	IL-2 (2.0 MIU / m^2), BID	Nothing	Nothing
Day 6	Nothing	Nothing	Nothing	Nothing	Nothing	Nothing
Day 7	Nothing	Nothing	Nothing	Nothing	Nothing	Nothing

3.1.4.1 Histamine and IL-2 Doses and Regimens.

Traditional dose response studies were not performed for either IL-2 or Histamine Dihydrochloride Injection in human patients with metastatic melanoma. The response assessed in the clinical trials in metastatic melanoma was duration of survival, which entails a lengthy clinical trial and follow-up procedure. Moreover, no surrogate endpoint(s) has been proven in metastatic melanoma which correlate(s) with an improvement in survival. For this reason the sponsor established the dose and schedule to be used in the pivotal clinical trials from previous experience and by the rationale described below. The Division of Oncologic Drug Products was consulted on the dose selection rationale and indicated basic agreement with the rationale for dose selection.

For this study, the doses and regimens for IL-2 were chosen based on several factors. There are at least three known receptors for IL-2 with differing ligand affinities and biological effects. The receptors are formed by a combination of various multimeric subunits including IL-2Rβ (CD122), IL-2Rα (CD25), and a γ chain (CD132). Expression of

the γ subunit with the β subunit forms an intermediate-affinity receptor (K_d ~ 1 n*M*) thought to be involved in NK- and T-cell cytotoxicity; coexpression of all three subunits forms a high-affinity receptor (K_d ~ 30 p*M*) thought to be involved in NK- and T-cell proliferation. Based on published research, these doses and schedules of IL-2 will have significant biological activity. In particular, the 2.0 MIU/m^2 regimen of IL-2 given during Weeks 2 and 4 was selected to target both high-affinity and intermediate-affinity receptors, whereas the 9.0 MIU/m^2 dose given during Weeks 1 and 3 was selected to target primarily intermediate-affinity receptors on NK cells.

The prescribed dose and regimen of histamine were intended to bind with all available histamine type-2 receptors (H_2Rs) while minimizing adverse reactions. Hellstrand *et al.*[96] have shown that the inhibitory effect on phagocyte-derived reactive oxygen metabolites (ROMs) is mediated strictly by H_2Rs. Research on hydrochloric acid secretion by the gastric mucosa, used as an example of activation of H_2Rs, has shown some divergence in terms of the maximally effective dose.[82,83] The work of Christiansen *et al.*[83] determined that even when histamine was administered for several hours, it was still the 15 – 45 minute period following the start of administration, similar to that of the augmented histamine test with subcutaneous stimulation, that offered a comparable estimate of the calculated maximal acid secretory capacity. In a dose-response study, the maximal acidity and maximal output were seen with doses of 28-29 µg/kg/hr body weight. However, maximal volume and maximal acidity were seen between 15 – 45 minutes when a total dose range of 7.5 to 22.5µg/kg would have been administered. This compares to our experience using 14-15 µg/kg SC to occupy available receptors during a 10-20 minute injection. However no details are available in regard to safety and side-effects experienced by the patient volunteers in the studies discussed above.[82,83] Further work by Adam *et al.*[84] found maximal acid responses with histamine doses at 20µg/kg.

Therefore, the sponsor chose a dose of 1.0 mg of histamine dihydrochloride given twice daily (which approximates to 14.3 ìg/kg for a 70 kg subject) to activate and saturate available H_2Rs on phagocytes. Research by Hellstrand *et al.*[85] showed that monocytes respond to histamine with an ED_{50} of 1-2 ìM and work by Lanas *et al.*[86] showed that maximal secretion for gastric mucosa is achieved at an equivalent dose of 2 ìM. Administration of 1mg of histamine using a twice daily (BID) regimen is supported by research[83] suggesting that H_2R activation, as measured by maximal acid output, may continue for three to five hours. Work published by Bury *et al.*[87] demonstrates that IV, SC or inhaled histamine significantly decreases neutrophil chemotaxis for four to eight hours, also via stimulation of

H_2Rs. Taken together, these data suggest that activation of the H_2Rs may last for four hours and inhibit the generation of ROMs for a similar period of time during cytokine administration. BID dosing would then appear appropriate to allow for safe and effective protection of NK and T-cells, and yet, allow phagocytes to remain effective against infectious agents during the intervals when the H_2R are less than fully saturated.

Timing of drug administration in relation to meals was not specified. The dose of IL-2 was always administered before the dose of histamine, and an interval of at least 6 hours between BID doses was recommended.

3.1.5 Statistical Analysis Plan

The original study protocol, finalized by the sponsor and submitted to the FDA, Division of Oncologic Drug Products (DODP) on July 1, 1997 (prior to any patient enrollment) specified that the final analysis plan would assess the effect of treatment on the two distinct populations of patients, defined below.

- The Intent to Treat Population, comprised of all randomized patients (also referred to as the ITT population).

- The Intent-to-Treat Population of patients with liver metastases at the time of entry into the study (also referred to as the ITT-LM population).

Further refinements of the statistical analysis plan were submitted and discussed with DODP and the final plan was approved on December 17, 1999. The statistical analysis plan was approved prior to the cut-off date for survival follow-up and prior to any study data being available for analysis (other than safety reports). A copy of the approved statistical analysis plan is submitted in Appendix 1.

A Drug Safety Monitoring Board (DSMB) had access to patient data as the study was underway. Monthly reports, summarizing safety and at one time, efficacy endpoints, were submitted to the DSMB by Covance; the reports were kept strictly confidential and were never forwarded to the sponsor.

3.1.5.1 Endpoints

The primary endpoint was survival, an unambiguous gold standard of efficacy. The analysis was conducted on survival information on all patients through March 8, 2000. There were no patients lost to follow-up at the 12-month follow-up point. Secondary endpoints included time to disease progression, time to treatment failure, tumor response, quality of life measurements, and safety.

3.1.5.2 Multiple Comparison Procedure

The central feature of the statistical analysis plan for the primary endpoint was the application of a multiple comparison procedure for the testing of two null hypotheses within the same experimental framework. In study M01 the two hypotheses were declared as follows:

> ***Null Hypothesis No. 1:*** Histamine Dihydrochloride for Injection, 1 mg/mL, given by subcutaneous injection in conjunction with Interleukin-2 does not improve the duration of survival of patients with advanced malignant melanoma compared to treatment with Interleukin-2 alone.
>
> ***Null Hypothesis No. 2****:* Histamine Dihydrochloride for Injection, 1 mg/mL, given by subcutaneous injection in conjunction with Interleukin-2 does not improve the duration of survival of patients with advanced malignant melanoma who have liver metastases at study entry compared to treatment with Interleukin-2 alone.

The statistical procedure known as the Holm-Šidák method (or Sharper Bonferroni method) was specified in the final statistical analysis plan. The Holm-Šidák method utilizes a technique called the family-wise error rate. The term "family-wise" refers to a family of related hypotheses, as is the case in study M01. The two hypotheses in the M01 study are actually the same but are applied to different populations. The family-wise error rate is said to be controlled if it can be predicted that, within a family of hypotheses, not more than one will be falsely rejected. The perfect multiple comparison procedure would control the family-wise type 1 error rate (risk of false-positive inference) so that it never exceeded $p = 0.05$ and, at the same time, would minimize the type 2 error rate (risk of false-negative inference). Unfortunately, these two goals are usually somewhat incompatible, but within the framework of biomedical research the most important goal is to avoid false positive inferences, i.e. to pursue a conservative approach.

Simple division of the p-value by the number of tests (i.e. $p' = p/m$, where m is the number of hypotheses – equivalent to meeting a $p \leq 0.025$ criteria for 2 tests) is too harsh when the hypotheses are logically or statistically correlated as they are in study M01. Such a calculation is performed with the classic Bonferroni method. The Holm-Šidák adjustment of the classic Bonferroni method adjusts the

p-values that result from the individual tests when multiple tests of hypotheses are conducted.

In the Holm-Šidák procedure one calculates the raw p-values that result from testing the individual hypotheses by conventional procedures (e.g. the log rank test for calculation of survival) and then:

Arrange the raw p-values in ascending order.

Start with the smallest p-value and calculate p' as $1-(1-p)^{m}$.

Proceed to the next smallest p-value and calculate p" as $1-(1-p)^{m-1}$.

3.1.5.3 Cox Regression Model

Covariates including known prognostic variables or demographic characteristics such as geographical location of the study site, age, sex, race, patient's metastatic disease sites at first evaluation, number of disease sites, prior anti-cancer therapies, WHO performance status, duration of prior therapy to randomization, LDH, and prior chemotherapy along with treatment group were assessed in conjunction with the primary efficacy endpoint.

3.1.6 Quality-of-Life Analysis Plan

A quality-of-life analysis plan was submitted to DODP on March 3, 2000 (copy provided in Appendix 2), prior to any quality-of-life data becoming available. The primary instrument for assessment of quality-of-life was the Quality-of-Well-Being, Self Administered scale (QWB-SA, ver. 1.04).

4. Results

4.1 Enrollment and Patient Demographics

Three hundred five (305) patients were randomized and enrolled into Study M01 at 56 institutions in the United States. Thus, the Intent-to-Treat (ITT) population is comprised of 305 patients. One hundred twenty-nine (42.3%) of the patients had liver metastases upon randomization and entry and therefore 129 patients comprise the Intent-to-Treat/Liver Mets (ITT-LM) population.

The demographics of study M01 are summarized in Table 12.

Table 12. Demographics and Baseline Characteristics of Patients in Study M01

Characteristic	Intent-to-Treat Population		Intent-to-Treat/Liver Mets Population	
	IL-2 Alone (N = 153)	IL-2 plus Histamine (N = 152)	IL-2 Alone (N = 74)	IL-2 plus Histamine (N = 55)
Sex				
Male	99 (64.7%)	90 (59.2%)	46 (62.2%)	27 (49.1%)
Female	54 (35.3%)	62 (40.8%)	28 (37.8%)	28 (50.9%)
Age [Mean (± SD)]	56.3 (± 13.12)	53.6 (± 13.79)	57.6 (± 13.13)	53.7 (± 14.37)
Median	56	53	58	53
Min-Max	21 – 89	22 – 84	25 – 88	31 – 79
< 65	103 (67.3%)	117 (77.0%)	46 (62.2%)	42 (76.4%)
≥ 65	50 (32.7%)	35 (23.0%)	28 (37.8%)	13 (23.6%)
Race				
Caucasian	147 (96.1%)	148 (97.4%)	71 (95.9%)	54 (98.2%)
Black	0 (0.0%)	1 (0.7%)	0 (0.0%)	1 (1.8%)
Other	6 (3.9%)	3 (2.0%)	3 (4.1%)	0 (0.0%)
WHO Performance Status				
PS 0 (KPS[a] 100 – 90)	103 (67.3%)	103 (68.2%)	44 (59.5%)	35 (63.6%)
PS 1 (KPS 80 – 70)	50 (32.7%)	48 (31.8%)	30 (40.5%)	19 (34.5%)
Disease Sites[b]				
Skin	40 (26.1%)	47 (30.9%)	18 (24.3%)	12 (21.8%)
Lymph Node	83 (54.2%)	77 (50.7%)	38 (51.4%)	24 (43.6%)
Bone	11 (7.2%)	19 (12.5%)	8 (10.8%)	5 (9.1%)
Lung	90 (58.8%)	99 (65.1%)	47 (63.5%)	32 (58.2%)
Liver	74 (48.4%)	55 (36.2%)	74 (100.0%)	55 (100.0%)
CNS	10 (6.5%)	12 (7.9%)	6 (8.1%)	1 (1.8%)
Other (spleen, adrenal, renal, GI)	76 (49.7%)	62 (40.8%)	37 (50.0%)	22 (40.0%)
Prior Chemotherapy				
No	115 (75.2%)	112 (73.7%)	53 (71.6%)	45 (81.8%)
Yes	38 (24.8%)	40 (26.3%)	21 (28.4%)	10 (18.2%)

Table 12. Demographics and Baseline Characteristics (continued)

Characteristic	Intent-to-Treat Population		Intent-to-Treat/Liver Mets Population	
	IL-2 Alone (N = 153)	IL-2 plus Histamine (N = 152)	IL-2 Alone (N = 74)	IL-2 plus Histamine (N = 55)
Number of Disease Sites				
1	31 (20.3%)	37 (24.3%)	7 (9.5%)	13 (23.6%)
2	47 (30.7%)	48 (31.6%)	17 (23.0%)	12 (21.8%)
> 2	75 (49.0%)	67 (44.1%)	50 (67.6%)	30 (54.5%)
Mean (± SD)	2.7 (± 1.43)	2.7 (± 1.71)	3.3 (± 1.50)	3.1 (± 2.02)
Time Since First Diagnosis of Primary Disease[c]				
0 – 2 years	64 (41.8%)	49 (32.2%)	27 (36.5%)	11 (20.0%)
3 – 4 years	37 (24.2%)	42 (27.6%)	21 (28.4%)	18 (32.7%)
> 4 years	47 (30.7%)	54 (35.5%)	25 (33.8%)	22 (40.0%)
Unknown	5 (3.3%)	7 (4.6%)	1 (1.4%)	4 (7.3%)
Mean (± SD)	4.4 (± 5.98)	4.5 (± 4.54)	5.0 (± 7.08)	5.2 (± 4.46)
Number of Prior Anti-Cancer Therapies				
Mean (± SD)	4.0 (± 2.38)	4.5 (± 2.91)	3.9 (± 2.36)	3.9 (± 2.87)
Median	4	4	3	3
Min – Max	0 – 13	0 – 15	0 - 13	0 – 15
LDH (U/L)				
N	143	144	68	51
Mean (± SD)	400.8 (± 526.05)	405.8 (± 583.41)	514.8 (± 684.91)	498.7 (± 512.95)
Median	200	191	261	279
Min – Max	97 – 4296	90 – 5430	97 - 4296	101 – 2141
< ULN	86 (60.1%)	92 (63.9%)	30 (44.1%)	19 (37.3%)
≥ ULN	57 (39.9%)	52 (36.1%)	38 (55.9%)	32 (62.7%)

[a] KPS = Karnofsky Performance Score.
[b] A patient may have more than one disease site. Thus, the sum of the percentages may be more than 100%.
[c] Interval (in years) since the first diagnosis of primary disease to the first dose of study medication.

4.2 Duration of Survival

The results of study M01 demonstrate that the adjunctive use of Histamine Dihydrochloride for Injection improves the duration of survival in patients suffering metastatic melanoma. The duration of survival was greater in patients receiving histamine dihydrochloride plus IL-2 compared to IL-2 alone in both primary analysis populations, but the effect was shown to be statistically significant only in the population of patients with liver metastases (adjusted $p = 0.0080$). Accordingly, the sponsor proposes that the new drug should be indicated for patients with melanoma that has metastasized to the liver.

The survival results are summarized in Table 13 for both primary efficacy populations. Kaplan-Meier survival distribution curves are found in Figures 3 and 4.

Figure 3. Proportion of Patients Surviving vs. Time for All Randomized Patients in Study M01
Histamine + IL-2 vs. IL-2 Alone

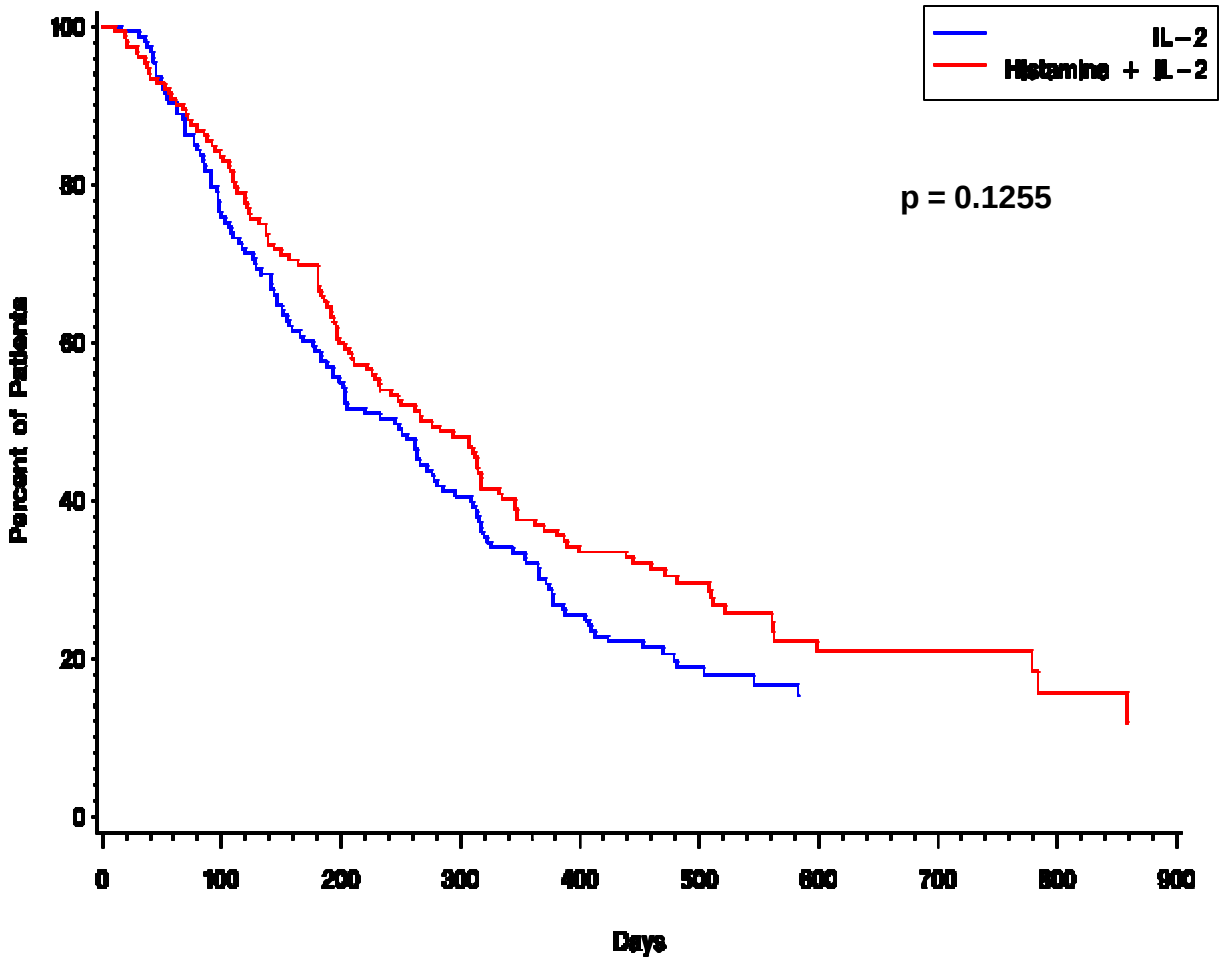


Figure 4. Proportion of Patients Surviving vs. Time for All Randomized Patients with Liver Metastases in Study M01
Histamine + IL-2 vs. IL-2 Alone

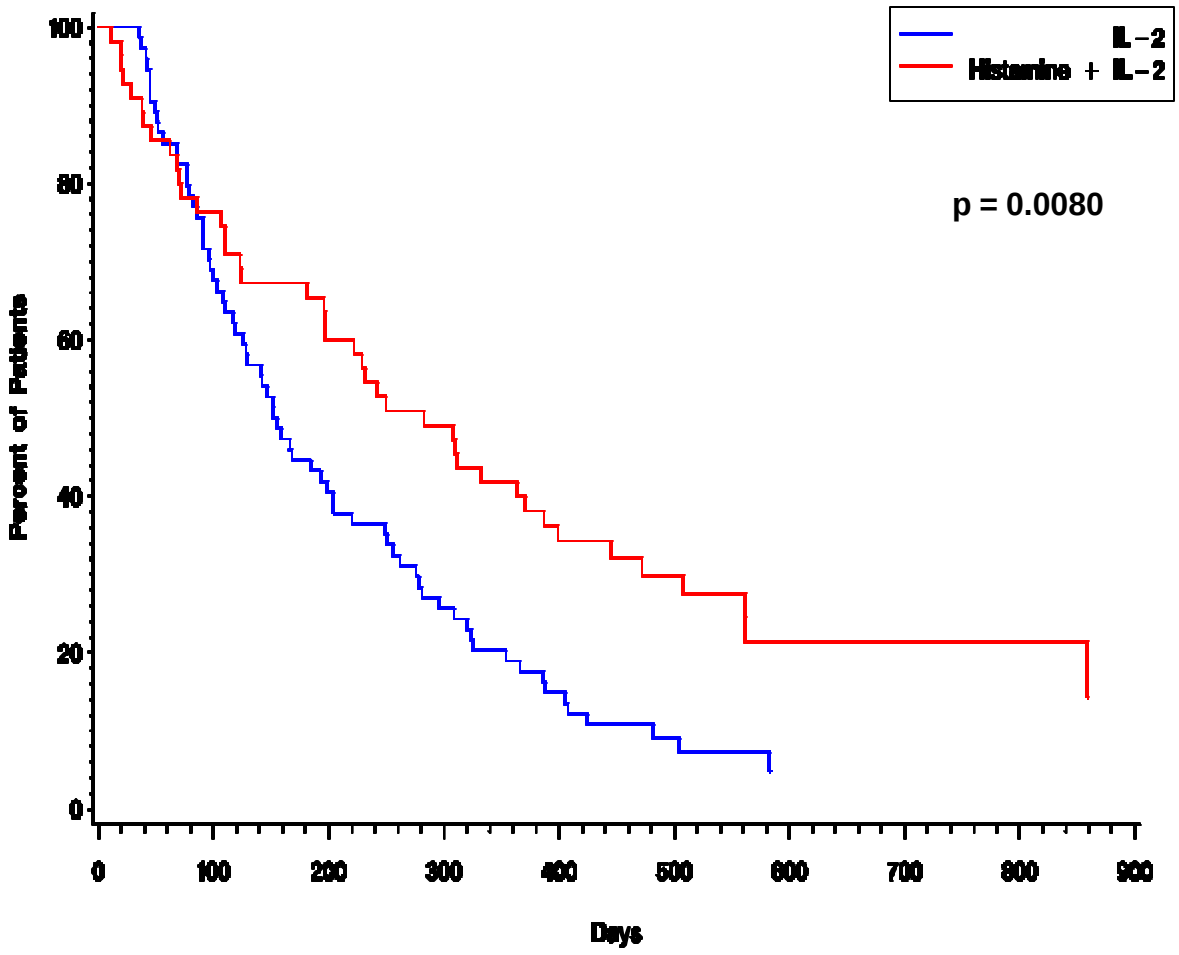


Table 13. Duration of Survival (Days) in Study M01 Through March 8, 2000

Population	Histamine + IL-2	IL-2 Alone	p-value[a]	Adjusted p-value[b]
All Randomized Patients with Liver Metastases Median (95% CI)	283 (197 - 387) (N = 55)	154 (119 – 204) (N = 74)	0.0040	0.0080
All Randomized Patients Median (95% CI)	272 (211 - 318) (N = 152)	245 (184 – 281) (N = 153)	0.1255	0.1255

[a] Raw p-value from log rank test
[b] p-value calculated by the Holm-Šidák (Sharper Bonferroni) method adjusting for multiplicity (m = 2)

In the ITT population the result shows a clear suggestion of improved survival with histamine but does not reach statistical significance (p = 0.1255, log rank test).

Median survival was increased by 129 days (4.3 months, 84%) in the population comprised of all randomized patients with liver metastases at baseline. The increase was highly statistically significant (p = 0.0080, log rank test) in this population.

It is interesting to speculate on why the addition of histamine to subcutaneous IL-2 provides such a remarkable benefit to patients with liver involvement. Our original hypothesis was focused on the fact that the population having liver metastases is more homogeneous as opposed to the ITT population and, thus, may allow for improved detection of a histamine effect. Once melanoma has metastasized to the liver, it becomes a dominant driver and predictor of outcome. Patients with liver metastases typically live 4-5 months and do not respond to any known existing treatment.

However, based on the known mechanism of action for histamine, it is also reasonable to consider the microenvironment of the liver and how it may contribute to a poorer prognosis and lack of a response to treatment. The liver is a necessary organ for live, and is a part of the reticular endothelial system. As such, it contains large numbers of lymphocytes including T-cells, B-cells, NK cells and a specialized NK cell with T-cell characteristics. The liver also has abundant monocytes and macrophages, including the resident macrophages called Kupffer cells. These cells have been implicated in generating significant oxidative stress (ROM production) observed in the liver following various insults including metastatic disease. It would make sense that a treatment that could reduce the oxidative stress would allow for better activation of the abundant lymphocytes resulting in a better immune response to the metastatic tumors.

As mentioned, this is a hypothetical speculation and experiments to prove this hypothesis specifically in the liver are ongoing in various laboratories.

4.3 Cox Regression Model

A univariate analysis of the relation between pretreatment factors and treatment outcome and an assessment of the independent pretreatment factors for survival was performed using the Cox Regression Model. In Tables 15 through 23 the results of the Cox Regression Model testing of covariates are summarized for both populations. The Cox Regression Model showed that treatment effect was highly significant when all covariates (multivariate) were included in the model ($p = 0.0017$) in the liver metastases population, but not for the population comprised of all randomized patients ($p = 0.0619$).

Interestingly, neither age alone nor gender alone were significant predictors of survival in either population in this study. Male sex and age ≥ 65 both exhibited hazard ratios > 1.000, but the Wald Chi-Square test did not indicate statistical significance.

Independent variables that were determined to be predictors of survival in the intent-to-treat population in this study were LDH (≥ ULN), number of disease sites > 1, metastatic disease in lymph nodes, bone, liver, and "other" sites (adrenal, kidney, spleen, GI), and performance status 1.

Table 14-A. Cox's Proportional Hazard Model
Survival from Day of Randomization for All Randomized Patients

Covariate	Hazard Ratio	95% CI	p-value[a]
Treatment (Histamine plus IL-2 vs. IL-2)	0.821	0.638 – 1.057	0.1261

[a] p-value is from the Wald Chi-Square test.

Table 14-B. Cox's Proportional Hazard Model
Survival from Day of Randomization for All Randomized Patients with Liver Metastases

Covariate	Hazard Ratio	95% CI	p-value[a]
Treatment (Histamine plus IL-2 vs. IL-2)	0.567	0.384 – 0.839	0.0045

[a] p-value is from the Wald Chi-Square test.

Table 15-A. Cox's Proportional Hazard Model Adjusting for Age
Survival from Day of Randomization for All Randomized Patients

Covariate	Hazard Ratio	95% CI	p-value[a]
Treatment (Histamine plus IL-2 vs. IL-2)	0.825	0.641 – 1.062	0.1352
Age (≥ 65 vs. < 65)	1.177	0.894 – 1.549	0.2465

[a] p-value is from the Wald Chi-Square test.

Table 15-B. Cox's Proportional Hazard Model Adjusting for Age
Survival from Day of Randomization for All Randomized Patients with Liver Metastases

Covariate	Hazard Ratio	95% CI	p-value[a]
Treatment (Histamine plus IL-2 vs. IL-2)	0.571	0.386 – 0.845	0.0051
Age (≥ 65 vs. < 65)	1.094	0.735 – 1.627	0.6579

[a] p-value is from the Wald Chi-Square test.

Table 16-A. Cox's Proportional Hazard Model Adjusting for Gender
Survival from Day of Randomization for All Randomized Patients

Covariate	Hazard Ratio	95% CI	p-value[a]
Treatment (Histamine plus IL-2 vs. IL-2)	0.824	0.641 – 1.061	0.1334
Sex (Male vs. Female)	1.232	0.947 – 1.602	0.1208

[a] p-value is from the Wald Chi-Square test.

Table 16-B. Cox's Proportional Hazard Model Adjusting for Gender
Survival from Day of Randomization for All Randomized Patients with Liver Metastases

Covariate	Hazard Ratio	95% CI	p-value[a]
Treatment (Histamine plus IL-2 vs. IL-2)	0.575	0.388 – 0.852	0.0058
Sex (Male vs. Female)	1.163	0.795 – 1.700	0.4369

[a] p-value is from the Wald Chi-Square test.

Table 17-A. Cox's Proportional Hazard Model Adjusting for LDH Value
Survival from Day of Randomization for All Randomized Patients

Covariate	Hazard Ratio	95% CI	p-value[a]
Treatment (Histamine plus IL-2 vs. IL-2)	0.823	0.634 – 1.069	0.1437
LDH (≥ ULN vs. < ULN)	2.604	1.991 – 3.405	0.0001

[a] p-value is from the Wald Chi-Square test.

Table 17-B. Cox's Proportional Hazard Model Adjusting for LDH Value
Survival from Day of Randomization for All Randomized Patients with Liver Metastases

Covariate	Hazard Ratio	95% CI	p-value[a]
Treatment (Histamine plus IL-2 vs. IL-2)	0.516	0.340 – 0.783	0.0018
LDH (≥ ULN vs. < ULN)	2.390	1.577 – 3.622	0.0001

[a] p-value is from the Wald Chi-Square test.

Table 18-A. Cox's Proportional Hazard Model Adjusting for Number of Disease Sites Survival from Day of Randomization for All Randomized Patients

Covariate	Hazard Ratio	95% CI	p-value[a]
Treatment (Histamine plus IL-2 vs. IL-2)	0.842	0.654 – 1.083	0.1808
Number of Disease Sites (1 vs. >1)	0.640	0.466 – 0.880	0.0059

[a] p-value is from the Wald Chi-Square test.

Table 18-B. Cox's Proportional Hazard Model Adjusting for Number of Disease Sites Survival from Day of Randomization for All Randomized Patients with Liver Metastases

Covariate	Hazard Ratio	95% CI	p-value[a]
Treatment (Histamine plus IL-2 vs. IL-2)	0.566	0.373 – 0.858	0.0074
Number of Disease Sites (1 vs. >1)	1.010	0.573 – 1.778	0.9739

[a] p-value is from the Wald Chi-Square test.

Table 19-A. Cox's Proportional Hazard Model Adjusting for Lymph Node Disease Site Survival from Day of Randomization for All Randomized Patients

Covariate	Hazard Ratio	95% CI	p-value[a]
Treatment (Histamine plus IL-2 vs. IL-2)	0.837	0.651 – 1.078	0.1679
Disease Site = Lymph Node (yes vs. no)	1.678	1.297 – 2.170	0.0001

[a] p-value is from the Wald Chi-Square test.

Table 19-B. Cox's Proportional Hazard Model Adjusting for Lymph Node Disease Site Survival from Day of Randomization for All Randomized Patients with Liver Metastases

Covariate	Hazard Ratio	95% CI	p-value[a]
Treatment (Histamine plus IL-2 vs. IL-2)	0.602	0.406 – 0.893	0.0116
Disease Site = Lymph Node (yes vs. no)	1.701	1.160 – 2.494	0.0066

[a] p-value is from the Wald Chi-Square test.

Table 20-A. Cox's Proportional Hazard Model Adjusting for Bone Disease Site Survival from Day of Randomization for All Randomized Patients

Covariate	Hazard Ratio	95% CI	p-value[a]
Treatment (Histamine plus IL-2 vs. IL-2)	0.810	0.629 – 1.042	0.1012
Disease Site = Bone (yes vs. no)	1.833	1.218 – 2.759	0.0037

[a] p-value is from the Wald Chi-Square test.

Table 20-B. Cox's Proportional Hazard Model Adjusting for Bone Disease Site Survival from Day of Randomization for All Randomized Patients with Liver Metastases

Covariate	Hazard Ratio	95% CI	p-value[a]
Treatment (Histamine plus IL-2 vs. IL-2)	0.592	0.400 – 0.878	0.0091
Disease Site = Bone (yes vs. no)	3.366	1.824 – 6.210	0.0001

[a] p-value is from the Wald Chi-Square test.

Table 21-A. Cox's Proportional Hazard Model Adjusting for Liver Disease Site Survival from Day of Randomization for All Randomized Patients

Covariate	Hazard Ratio	95% CI	p-value[a]
Treatment (Histamine plus IL-2 vs. IL-2)	0.825	0.641 – 1.061	0.1341
Disease Site = Liver (yes vs. no)	1.476	1.146 – 1.901	0.0026

[a] p-value is from the Wald Chi-Square test.

Table 22-A. Cox's Proportional Hazard Model Adjusting for Other Disease Site Survival from Day of Randomization for All Randomized Patients

Covariate	Hazard Ratio	95% CI	p-value[a]
Treatment (Histamine plus IL-2 vs. IL-2)	0.840	0.652 – 1.082	0.1767
Disease Site = Other (yes vs. no)	1.379	1.070 – 1.776	0.0129

[a] p-value is from the Wald Chi-Square test.

Table 22-B. Cox's Proportional Hazard Model Adjusting for Other Disease Site Survival from Day of Randomization for All Randomized Patients with Liver Metastases

Covariate	Hazard Ratio	95% CI	p-value[a]
Treatment (Histamine plus IL-2 vs. IL-2)	0.588	0.397 – 0.871	0.0081
Disease Site = Other (yes vs. no)	1.304	0.895 – 1.900	0.1673

[a] p-value is from the Wald Chi-Square test.

Table 23-A. Cox's Proportional Hazard Model Adjusting for Baseline Performance Status Survival from Day of Randomization for All Randomized Patients

Covariate	Hazard Ratio	95% CI	p-value[a]
Treatment (Histamine plus IL-2 vs. IL-2)	0.809	0.628 – 1.042	0.1006
Baseline Performance Status (1 vs. 0)	2.354	1.804 – 3.070	0.0001

[a] p-value is from the Wald Chi-Square test.

Table 23-B. Cox's Proportional Hazard Model Adjusting for Baseline Performance Status Survival from Day of Randomization for All Randomized Patients with Liver Metastases

Covariate	Hazard Ratio	95% CI	p-value[a]
Treatment (Histamine plus IL-2 vs. IL-2)	0.517	0.347 – 0.772	0.0012
Baseline Performance Status (1 vs. 0)	2.306	1.553 – 3.424	0.0001

[a] p-value is from the Wald Chi-Square test.

The effect of all covariates taken together was also investigated in a Cox Regression Model. The results of the multivariate analyses (Tables 24 and 25) indicate that when all covariates are taken together there is little effect of the significance of the treatment effect.

Table 24. Cox's Proportional Hazard Model Adjusting for Covariates (Selected Baseline Characteristics) in Population Comprised of all Randomized Patients (N = 305)

Covariate	Hazard Ratio	95% CI	p-value
Treatment (Histamine + IL-2 vs. IL-2)	0.770	0.586 – 1.013	0.0619*
Race (Caucasian vs. All Other)	0.536	0.255 – 1.126	0.0998
Sex (Male vs. Female)	1.626	1.208 – 2.189	0.0013
Prior Chemotherapy (Yes vs. No)	1.185	0.854 – 1.645	0.3089
Prior anti-Cancer Therapy (Yes vs. No)	0.764	0.374 – 1.561	0.4602
LDH (≥ ULN vs. < ULN)	2.049	1.500 – 2.798	0.0001
Baseline Performance Status (1 vs. 0)	2.100	1.554 – 2.838	0.0001
Geographic Region			
Mid-West vs. South	1.213	0.743 – 1.978	0.4397
North vs. South	0.857	0.527 – 1.394	0.5350
West vs. South	0.731	0.469 – 1.139	0.1665
Disease Sites			
Skin (Yes vs. No)	1.371	0.977 – 1.923	0.0680
Lymph Node (Yes vs. No)	1.857	1.324 – 2.603	0.0003
Bone (Yes vs. No)	2.840	1.746 – 4.617	0.0001
Lung (Yes vs. No)	1.513	1.042 – 2.195	0.0293
Liver (Yes vs. No)	1.416	1.016 – 1.975	0.0401
CNS (Yes vs. No)	1.744	1.027 – 2.961	0.0397
Other (Yes vs. No)	1.508	1.056 – 2.154	0.0239
Number of Disease Sites			
(1 vs. >2)	1.872	0.935 – 3.746	0.0766
(2 vs. >2)	1.482	0.913 – 2.405	0.1111
Age (≥ 65 vs. < 65)	1.088	0.796 – 1.487	0.5974

ULN = Upper Limit of Normal
* The statistics given for treatment are for the multivariate model.

Table 25. Cox's Proportional Hazard Model Adjusting for Covariates (Selected Baseline Characteristics) in Population Comprised of all Randomized Patients with Liver Metastases (N = 129)

Covariate	Hazard Ratio	95% CI	p-value
Treatment (Histamine + IL-2 vs. IL-2)	0.463	0.286 – 0.750	0.0017*
Race (Caucasian vs. All Other)	0.698	0.200 – 2.445	0.5745
Sex (Male vs. Female)	1.203	0.769 – 1.884	0.4179
Prior Chemotherapy (Yes vs. No)	1.224	0.707 – 2.117	0.4703
Prior Anti-Cancer Therapy (Yes vs. No)	0.319	0.100 – 1.017	0.0535
LDH (≥ ULN vs. < ULN)	2.170	1.375 – 3.423	0.0009
Baseline Performance Status (1 vs. 0)	2.593	1.656 – 4.061	0.0001
Geographic Region			
Mid-West vs. South	1.180	0.519 – 2.684	0.6931
North vs. South	1.150	0.525 – 2.520	0.7267
West vs. South	1.028	0.502 – 2.101	0.9406
Disease Sites			
Skin (Yes vs. No)	1.452	0.839 – 2.512	0.1830
Lymph Node (Yes vs. No)	1.469	0.865 – 2.494	0.1549
Bone (Yes vs. No)	5.795	2.682 – 12.519	0.0001
Lung (Yes vs. No)	1.241	0.627 – 2.456	0.5358
CNS (Yes vs. No)	1.330	0.557 – 3.174	0.5212
Other (Yes vs. No)	1.058	0.630 – 1.778	0.8503
Number of Disease Sites			
(1 vs. >2)	1.996	0.619 – 6.439	0.2475
(2 vs. >2)	0.913	0.413 – 2.020	0.8222
Age (≥ 65 vs. < 65)	1.154	0.733 – 1.819	0.5364

ULN = Upper Limit of Normal

* The statistics given for treatment are for the multivariate model.

4.4 Disease Progression

Secondary endpoints of efficacy included time to progression for both the ITT and ITT-LM populations. Time to progression was measured as the number of days from randomization to the first observed response of progressive disease, or death due to melanoma. Time to Treatment Failure was defined as the date of the last response of observed progressive disease from week 12 or later, or death due to melanoma.

Figure 5 displays a Kaplan-Meier distribution curve comparing time to progression within the two treatment groups in the ITT population and Figure 6 displays the same type of curve for the time to treatment failure in the ITT population.

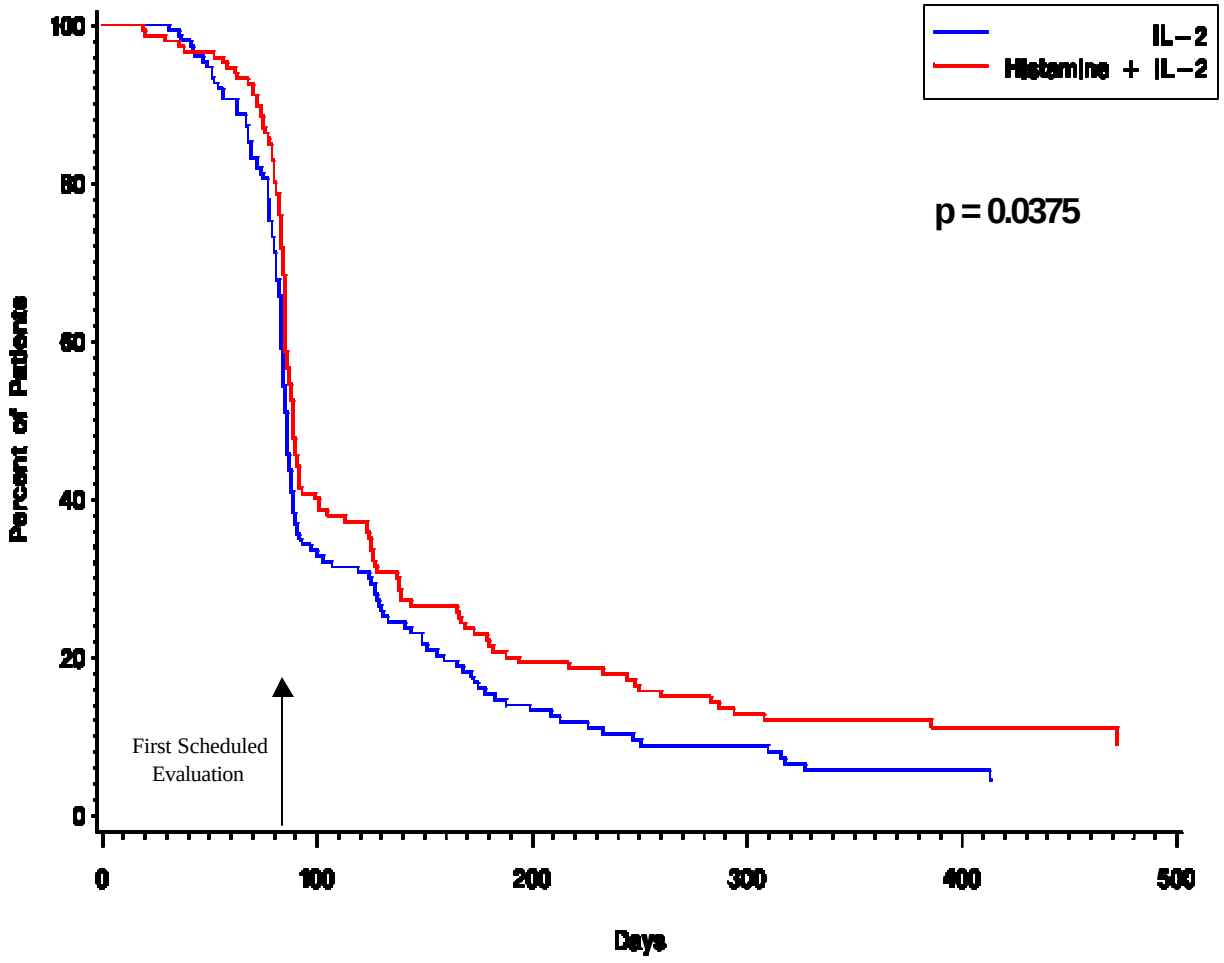


Figure 5. Time to Progression from Day of Randomization – All Patients Intent-to-Treat Population

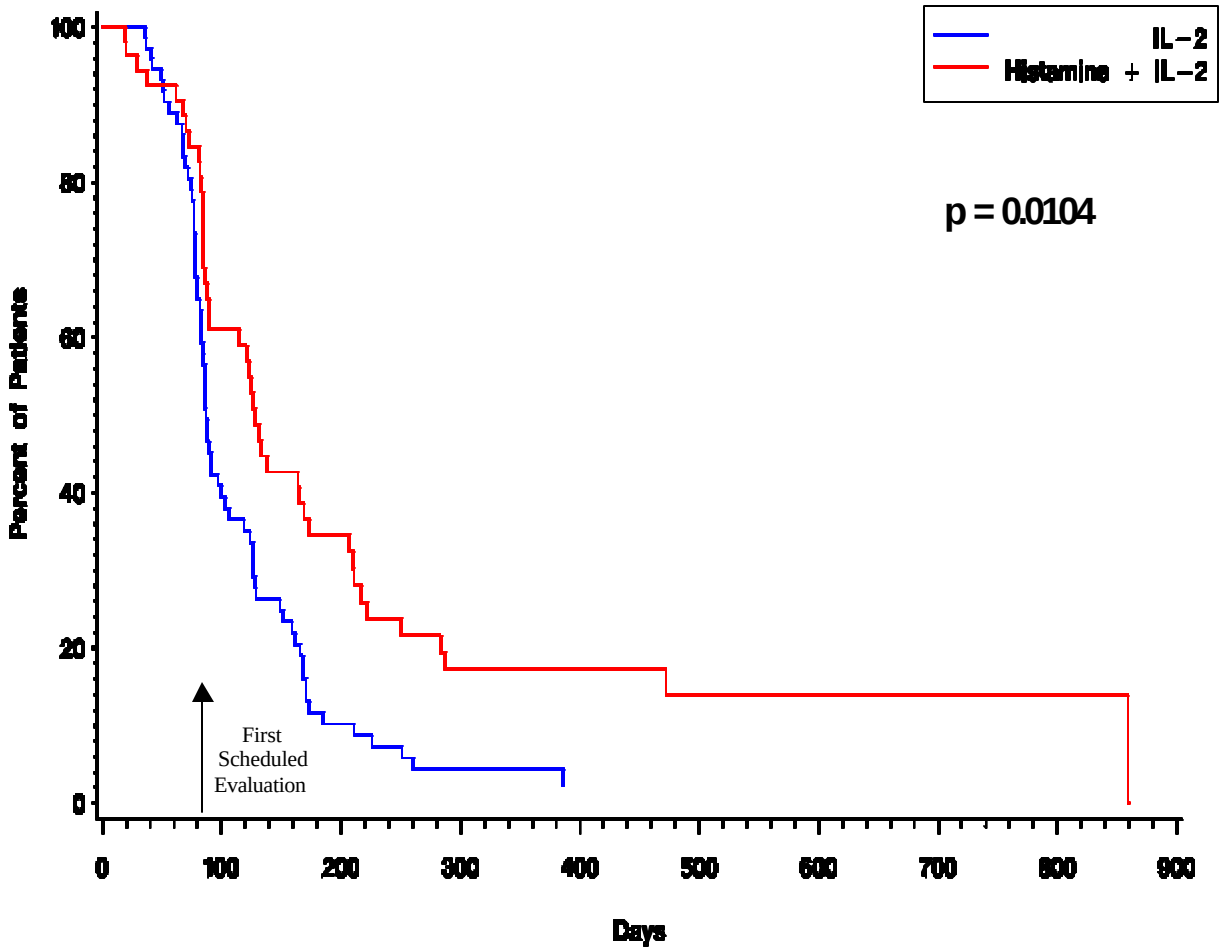


Figure 6. Time to Treatment Failure from Day of Randomization – All Patients Intent-to-Treat Population

Analysis of the data demonstrated a significant difference in favor of histamine + IL-2 for both time to progression and time to treatment failure (p = 0.0375 and p = 0.0104, respectively).

Figures 7 and 8 display the Kaplan-Meier curves for the time to progression and time to treatment failure in the ITT-LM population. Both time to progression and time to treatment failure were significantly increased by the addition of histamine (p = 0.0074 and p= 0.0033), respectively.

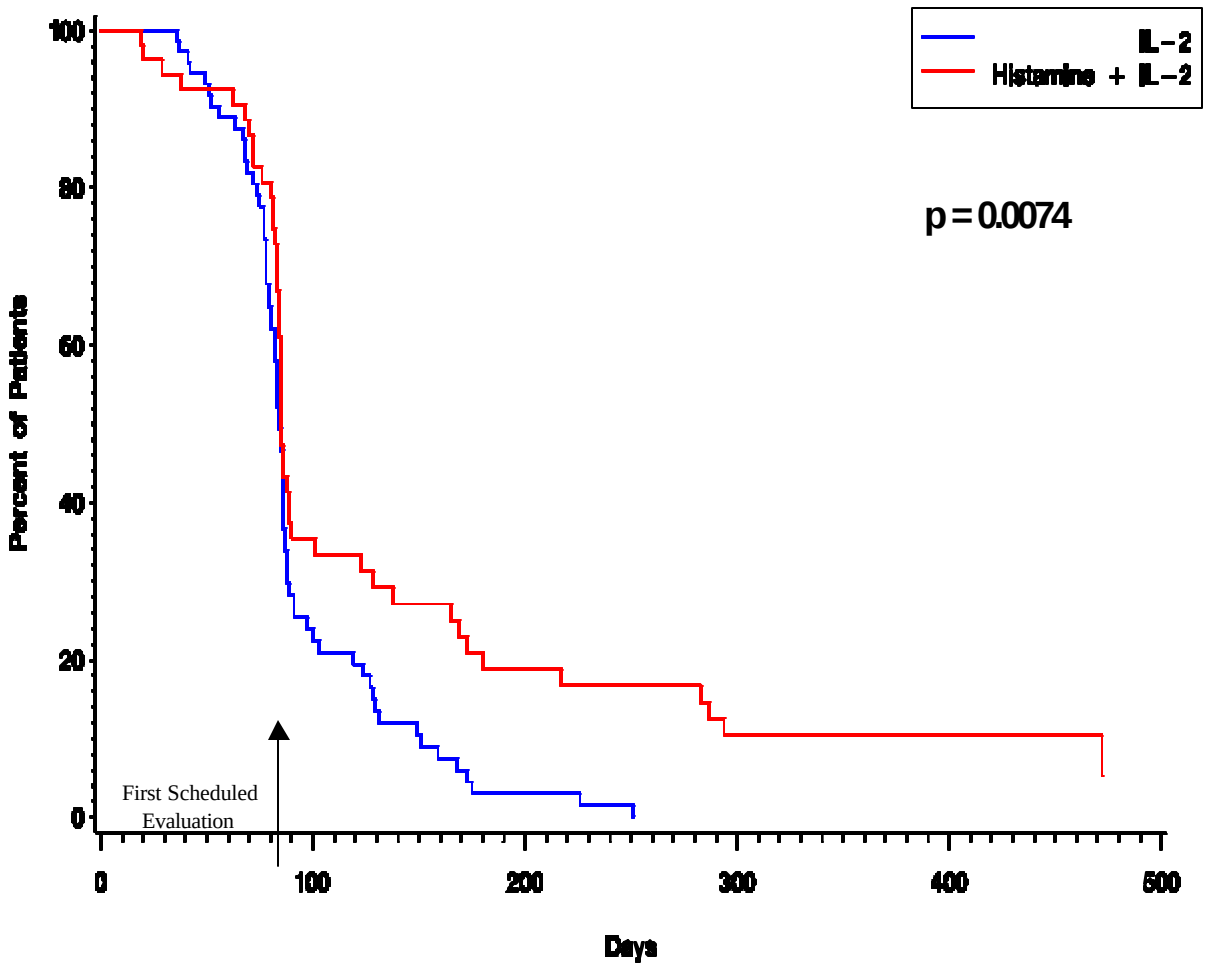


Figure 7. Time to Progression from Day of Randomization - Intent-to-Treat Population with Liver Metastases

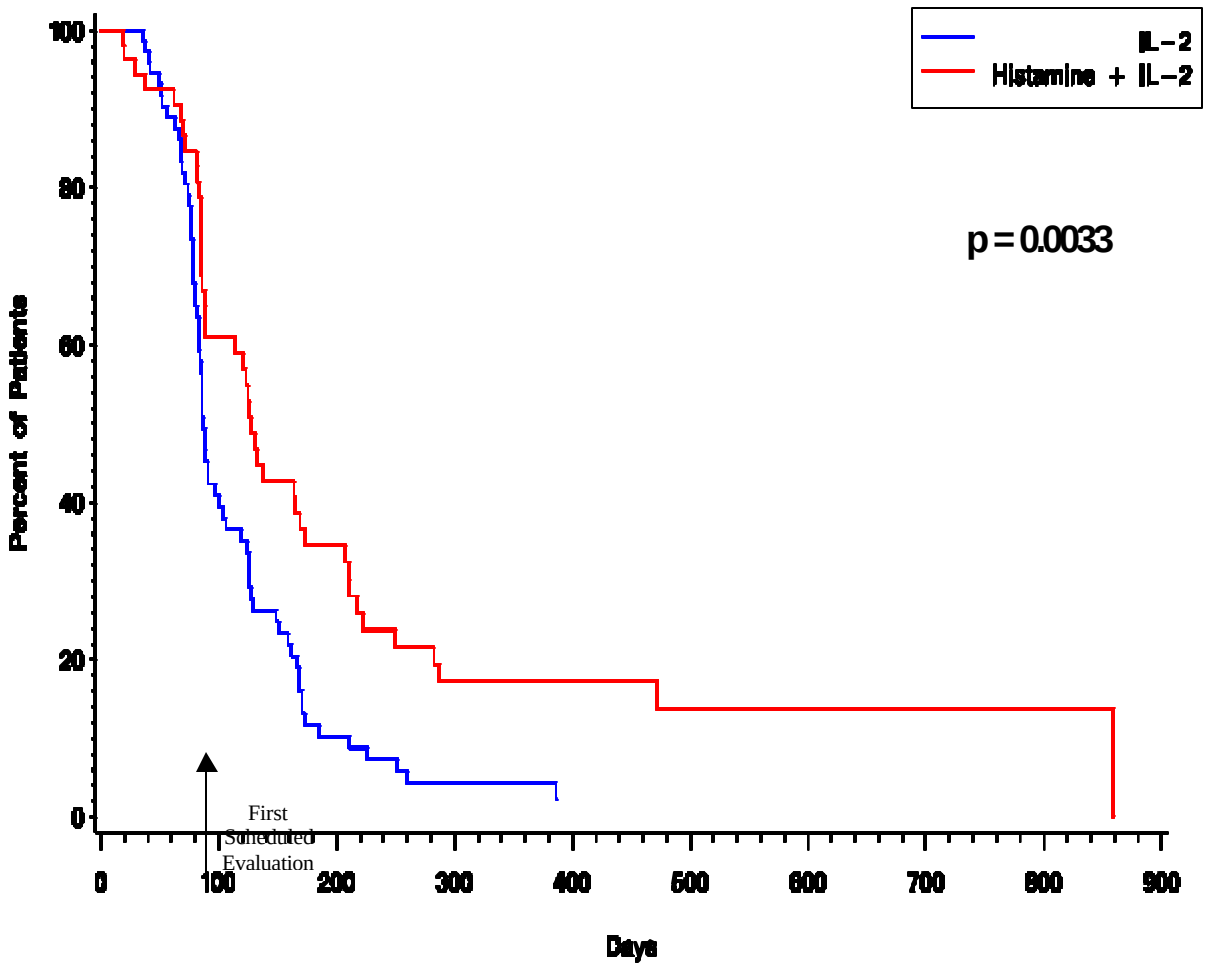


Figure 8. Time to Treatment Failure from Day of Randomization-Intent-to-Treat Population with Liver Metastases

4.5 Tumor Response

Tumor response was assessed in study M-01. In the ITT population, details of the best tumor response achieved by patients are given in Table 26.

Table 26. Best Tumor Response: ITT Population [a]

Best Confirmed Response[a]	Histamine + IL-2 (N = 152) n (%)[b]	IL-2 Alone (N = 153) n (%)[b]	p-value[c]
Patients with best tumor response assessed	109 (100.0%)	104 (100.0%)	
CR	1 (0.9%)	2 (1.9%)	
PR	4 (3.7%)	3 (2.9%)	
MR	7 (6.4%)	3 (2.9%)	
SD	29 (26.6%)	21 (20.2%)	
PD	68 (62.4%)	75 (72.1%)	
CR + PR	5 (4.6%)	5 (4.8%)	
CR + PR + MR + SD	41 (37.6%)	29 (27.9%)	0.1460
PD	68 (62.4%)	75 (72.1%)	

[a] ITT = Intent-to-Treat; CR = Complete Remission; PR = Partial Remission ; MR = Minimal Regression; SD = Stable Disease; PD = Progressive Disease

[b] Percentages are based upon the number of patients assessed in each treatment group (n).

[C] Based on Fisher's exact test, due to the small number of patients, for descriptive purposes only. Note that p-values reflect treatment by response 2x5 and 2x2 tables, respectively.

The numbers of patients in either treatment arm achieving a complete or partial remission was identical with 5 patients in each arm recording these responses. When the total of CR, PR, MR, and SD proportions are analyzed it is seen that the group receiving histamine plus IL-2 fares better than the group receiving IL-2 alone. Thus, there is a higher percentage of patients with "Lack of Disease Progression" within the histamine plus IL-2 group.

Within the ITT-LM population, details of the best tumor response achieved by patients is given in Table 27.

Table 27. Best Tumor Response: ITT-LM Population [a]

Best Confirmed Response[a]	Histamine + IL-2 (N = 37) n (%)[b]	IL-2 Alone (N = 46) n (%)[b]	p-value[c]
Patients with best tumor response assessed	37 (100.0%)	46 (100.0%)	
CR	0	0	
PR	2 (5.4%)	0	
MR	4 (10.8%)	2 (4.3%)	
SD	8 (21.6%)	7 (15.2%)	
PD	23 (62.2%)	37 (80.4%	
CR + PR	2 (5.4%)	0	
CR + PR + MR + SD	14 (37.8%)	9 (19.6%)	0.0854
PD	23 (62.2%)	37 (80.4%)	

[a] ITT = Intent-to-Treat Liver Metastases; CR = Complete Remission; PR = Partial Remission ; MR = Minimal Regression; SD = Stable Disease; PD = Progressive Disease

[b] Percentages are based upon the number of patients assessed in each treatment group.

[C] Based on Fisher's exact test, due to the small number of patients, for descriptive purposes only. Note that p-values reflect treatment by response 2x5 and 2x2 tables, respectively.

The number of patients in the histamine + IL-2 group (n = 2) achieving a complete or partial remission was greater than in the IL-2 alone group (n = 0). In the combined total of all the responses, apart from progressive disease (PD) Lack of Disease Progression, there was a higher percentage of patients in the histamine + IL-2 group (37.8%) than in the IL-2 alone group (19.6%; p = 0.0854). Lack of Disease Progression may become a more relevant assessment when testing cytostatic agents such as antiangiogenic factors, immunotherapeutics and vaccines. Stable disease can have significant benefit for patients and may allow for previously unavailable treatment options.

4.6 Quality-of-Life

Quality-of-Life was assessed in study M01 by use of a validated survey instrument. The survey included a self-administered Quality of Well-Being questionnaire (QWB-SA).

The QOL survey was administered prior to the start of therapy and at the end of each six-week cycle. The survey was not administered after discontinuation from the study. A brief explanation of each component of the survey is provided below.

- **QWB-SA.** The QWB-SA is a 76 item self-administered QOL questionnaire that focuses on mobility, physical activity, social activity, and symptoms or problems. The QWB-SA was chosen because it is a comprehensive utility-based measure that integrates morbidity and mortality into a common measurement unit. Among the validated utility-based measures, which include the 6-item EuroQol and the 7-item Health Utilities Index, the 76-item QWB-SA appears to be an appropriate choice for evaluating patients with advanced malignant melanoma because of its breadth of focus.

  The QWB-SA score ranges from 0.0, meaning death, to 1.0 meaning optimum functioning without symptoms. The scores represent measured community preference weights associated with each observed combination of morbidity, physical activity, social activity, and symptoms and is a self-administered version of a widely used and validated interviewer administered instrument.[88]

  The QOL analysis is comprised of two main components: 1) comparison of QOL scores of patients while on study therapy between treatment groups, and 2) integration of QWB-SA scores with survival and comparison of quality-adjusted survival time between groups. The QOL analysis was performed on both primary study populations for study M01, namely the Intent-to-Treat population comprised of all randomized patients and the Intent-to-Treat population comprised of all randomized patients with liver metastases at study entry. Data analyses were performed using SAS version 6.12 (SAS Institute, Cary, NC). Statistical significance was concluded using the 0.05 level of significance and two-tailed tests. Baseline demographic and clinical characteristics and QOL scores were compared between treatment groups using Student's t-test to compare continuous variables with normal distributions, a Mann-Whitney U test to compare continuous variables with skewed distributions, and a chi-square test to compare categorical variables. Any variables showing statistically significant differences between treatment groups at baseline were controlled for in the analysis of treatment effects on QOL outcomes. Study site and baseline variables that suggested a trend between treatment groups at the $p < 0.10$ level were included in the regression models to determine if they were significantly associated with treatment effects.

  A longitudinal data analysis (LDA) using the generalized estimating equations (GEE) approach was performed to estimate differences in the average QWB-SA scores over time between the treatment groups. Based on an assessment of the nature of the autocorrelation of QWB-SA scores between the different assessment time points, a random intercept linear regression model was specified, in which the QWB-SA score was regressed on time, treatment group, and the interaction of time and treatment group. The LDA model generates least squares means (LSMeans), (i.e., predicted mean scores) at each assessment time point by treatment group, adjusting for the other variables in the model. It can also generate predicted scores for each patient at each assessment time point. These patient-level predicted scores were used to calculate quality-adjusted survival time gained for each patient. Quality-adjusted

survival time was considered a secondary QOL endpoint and was calculated using the area under the curve of QWB-SA scores plotted versus time. Specifically, for each patient, quality-adjusted survival was calculated using the equation below.

Quality-adjusted survival was compared between treatment groups using a Mann-Whitney U test.

Calculation of Quality-Adjusted Survival for a Given Patient

$$\text{Score} = (t_i - t_{i-1}) \times [(Y_i + Y_{i-1}) / 2]$$

Where: t_i is time in days since randomization
Y_i is the predicted QWB-SA score
i = 0 to 8 (baseline through cycle 8)

Example:

If the patient's predicted QWB-SA scores at baseline and cycles 1 through 8 (timespan between assessments was 42 days) were: 0.62, 0.58, 0.54, 0.48, 0.43, 0.30, 0.0, 0.0, and 0.0, the quality-adjusted survival for this patient would be equal to:

[42 x (0.62 + 0.58) / 2] + [42 x (0.58 + 0.54) / 2] + [42 x (0.54 + 0.48) / 2] + [42 x (0.48 + 0.43) / 2] +[42 x (0.43 + 0.30) / 2] + [42 x (0.30 + 0.0) / 2] + [42 x (0.0 +0.0) / 2] + [42 x (0.0 +0.0) / 2] = **113.8 quality-adjusted days**.

Of the 305 patients randomized in study M01, 301 (98.7%) completed at least one QWB-SA questionnaire and thus comprised the intent-to-treat (ITT) population for the QOL analyses. Of these 301 ITT patients, 126 (41.9%) had liver metastases at baseline and thus comprised the ITT-liver metastases (ITT-LM) group.

4.6.1 QWB-SA Scores

Table 28 and Figure 9 summarize the predicted mean QWB-SA scores by treatment group generated using the LDA model while controlling for baseline differences in liver metastases. At baseline, the mean QWB-SA score for each group was approximately 0.60 and, as expected, because of the progressive nature of advanced malignant melanoma, declines over the follow-up period. Overall, the change in QWB-SA scores over time slightly favored the histamine plus IL-2 group, but did not attain statistical significance (p = 0.511, type III F test). Differences in QWB-SA scores

between groups appeared most substantial at the end of cycles 2, 3, 4, and 5, in which the differences were 0.06, 0.09, 0.07, and 0.06, respectively. The addition of site and age to the model did not have an impact on the findings.

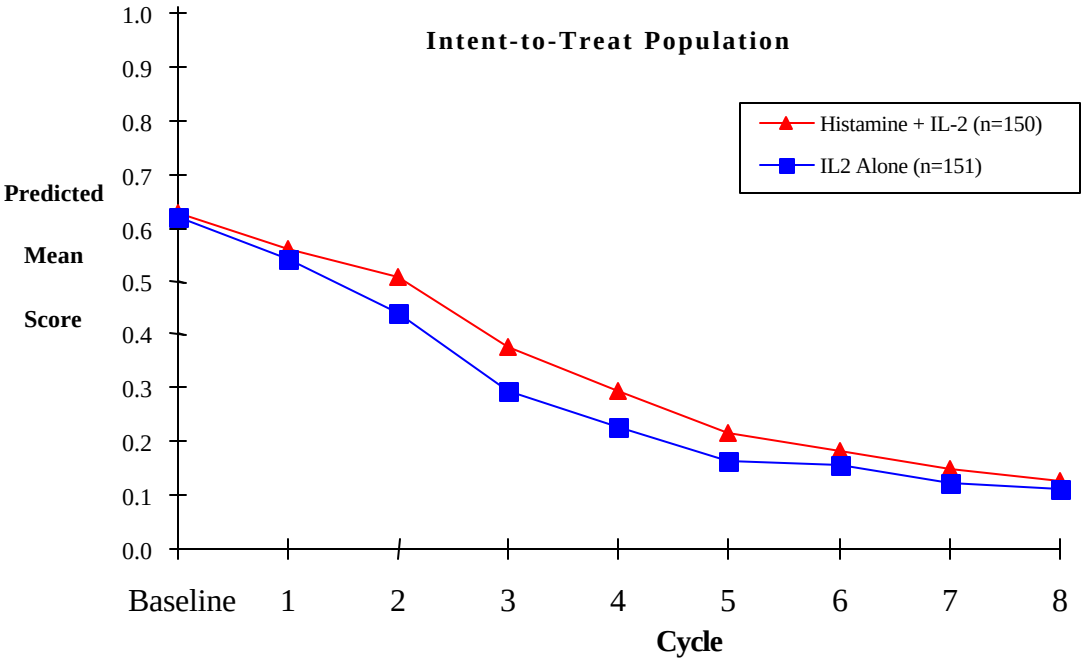
Similarly, in the population of patients with liver metastases, QWB-SA scores in the histamine plus IL-2 group were higher than those in the IL-2 alone group (Table 28 and Figure 9). Overall, there was evidence of a significant change in QWB-SA scores over time favoring the histamine plus IL-2 group ($p = 0.018$, type III F test). The differences favoring histamine plus IL-2 were statistically significant at the end of cycle 2 ($p = 0.011$), cycle 3 ($p = 0.002$), cycle 4 ($p = 0.044$), and cycle 5 ($p = 0.033$). The differences in predicted QWB-SA scores at these cycles ranged from 0.11 to 0.16. The addition of site and age to the model did not impact these findings.

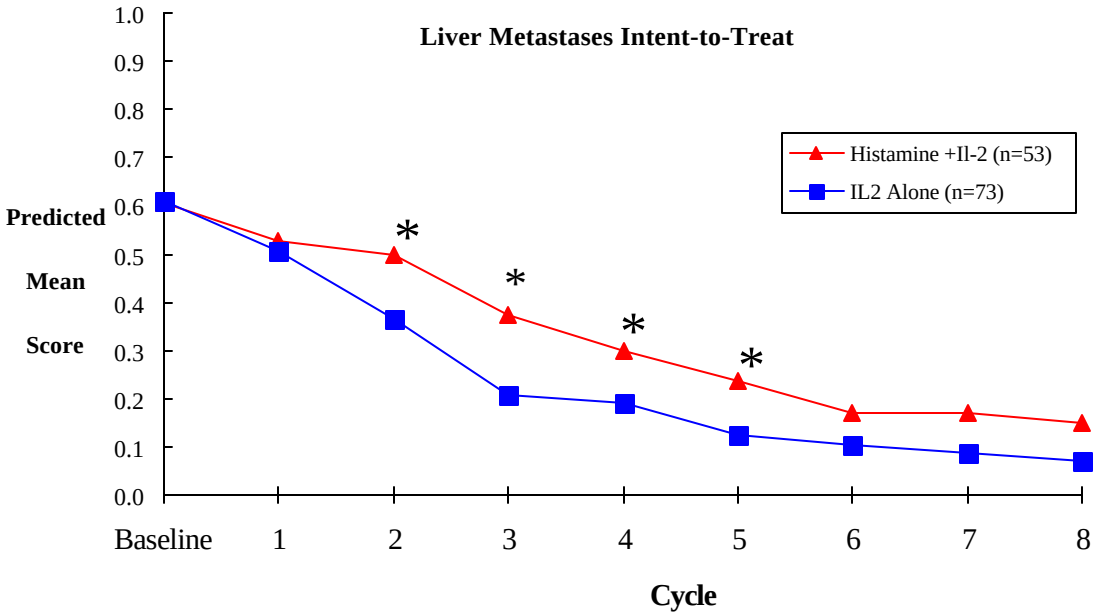
Table 28. Mean QWB-SA Score [LS Mean (SE)] (Adjusted) for All Randomized Patients and All Randomized Patients with Liver Metastases

Cycle	All Randomized Patients [a,b]				All Randomized Patients with Liver Metastases at Baseline [a,b]			
	IL-2 Alone (N = 151)	Histamine plus IL-2 (N = 150)	Difference[c]	p-value	IL-2 Alone (N = 73)	Histamine plus IL-2 (N = 53)	Difference[c]	p-value
Base	0.62 (0.01)	0.63 (0.01)	0.01	0.673	0.61 (0.02)	0.61 (0.02)	0.00	0.984
1	0.54 (0.02)	0.56 (0.02)	0.02	0.637	0.50 (0.03)	0.53 (0.03)	0.03	0.523
2	0.44 (0.03)	0.50 (0.03)	0.06	0.098	0.36 (0.04)	0.50 (0.05)	0.14	0.011*
3	0.29 (0.03)	0.38 (0.03)	0.09	0.065	0.21 (0.04)	0.37 (0.05)	0.16	0.002*
4	0.22 (0.03)	0.29 (0.03)	0.07	0.141	0.19 (0.04)	0.30 (0.05)	0.11	0.044*
5	0.16 (0.02)	0.22 (0.03)	0.06	0.226	0.12 (0.03)	0.24 (0.05)	0.12	0.033*
6	0.16 (0.02)	0.18 (0.03)	0.02	0.626	0.10 (0.03)	0.17 (0.04)	0.07	0.114
7	0.12 (0.02)	0.15 (0.02)	0.03	0.568	0.09 (0.02)	0.17 (0.04)	0.08	0.058
8	0.11 (0.02)	0.13 (0.02)	0.02	0.853	0.07 (0.02)	0.15 (0.04)	0.08	0.069

[a] Scores range from 0.0 to 1.0, with higher scores reflecting better QOL.
[b] Predicted mean score (LSMeans) and inferences (p-values) from random intercept linear regression model.
[c] Difference in predicted mean scores (LSMeans); Histamine plus IL-2 minus IL-2 alone.
* Statistically Significant

Figure 9. Predicted Mean QWB-SA Scores by Treatment Group





* p < 0.05 for difference between treatment groups

[a] Score range: 0.0 - 1.0, with higher scores reflecting better QOL

[b] Predicted mean scores (LSMeans) and p-values from random intercept linear regression model

4.6.2 Clinical Interpretation of QWB-SA Scores.

The QWB-SA score reflects the desirability of a health state. Both populations in study M01 exhibited QWB-SA scores of approximately 0.60 at baseline. This result is similar to a study that included terminal patients comprised of cancer and AIDS patients, who exhibited a mean QWB-SA score of 0.57.[89] In comparison, a mean QWB-SA score of 0.70 was observed in a population of community-dwelling individuals ≥ 65 years of age who were randomly selected from primary care physician's offices.[90]

Changes in QWB-SA scores as small as 0.04 have been found to be clinically meaningful. Specifically, a mean improvement of 0.04 points on the QWB scale was observed among patients treated for various musculoskeletal disorders, including osteoarthritis, rheumatoid arthritis, and back pain, who experienced a significant improvement on the Arthritis Impact Measurement Scale, which measures pain, physical functioning, and psychosocial functioning.[91] In another study of patients treated for cystic fibrosis, the patients showed a significant improvement in forced vital capacity and showed a mean improvement of 0.09 points on the QWB scale after treatment.[92]

QWB scores also have demonstrated expected trends across different diseases and clinical classification systems. Mean QWB scores of 0.71, 0.60, and 0.58 were observed among patients with non-insulin dependent diabetes mellitus, multiple sclerosis, and life threatening traumatic injury, respectively.[89]

4.6.3 Quality-Adjusted Survival

The predicted scores for each patient generated from the LDA models were used to calculate and compare quality-adjusted survival between treatment groups. Based on the imputed predicted QWB-SA scores, the findings showed significant differences in quality-adjusted survival in both populations. Specifically, for the population comprised of all randomized patients, the median quality-adjusted survival was 31.3 days longer in the histamine plus IL-2 group compared to the IL-2 alone group (105.6 days vs. 74.3 days, respectively; $p = 0.007$). For the population comprised of all randomized patients with liver metastases at baseline, the median quality-adjusted survival was 50.2 days longer in the histamine plus IL-2 group compared to the IL-2 alone group (113.0 days vs. 62.8 days, respectively; ($p = 0.010$). The results were essentially unchanged when the test was repeated without having imputed the predicted QWB-SA scores.

Overall, the length of quality-adjusted survival was substantially lower than actual observed survival in the study because of the diminished level of QOL at study entry. At baseline, the patient population had an average QWB-SA score of approximately 0.60. The QWB-SA score of 0.60 represents 60% of

optimal functioning (optimal functioning is recorded as a score of 1.0). When incorporated into a calculation of quality-adjusted survival, a score of 0.60 would reduce a period of optimal functioning by 40%. For example, if a patient had retained a score of 0.60 over 12 month's follow-up, the patient's quality-adjusted survival would be 0.60 x 356 days = 213.6 quality-adjusted days. Thus, because patients entered the study with QWB-SA scores of 0.60 and gradually worsened over time, their overall quality-adjusted survival is substantially lower than actual observed survival.

4.6.4 Quality-of-Life Conclusions.

The addition of an adjunct therapy administered as a slow subcutaneous injection twice daily 5 days/week for 4 weeks during a treatment cycle did not appear to adversely impact QoL. No significant differences were observed in patients' overall state of health and general health perceptions over the study period. Among patients who were alive and completed the overall state of health and general health perception items, QoL scores worsened shortly after treatment and subsequently improved over time. This finding is consistent with that observed in a recent study of patients with metastatic renal cell carcinoma who received inhaled IL-2 immuno-therapy, in which the authors suggested that the renal cell carcinoma patients seemed to adapt to the IL-2 therapy as shown by their QoL scores that initially worsened and subsequently improved to their pre-treatment levels by the third month of treatment.[93] Assessment of the quality-of-life data in study M01, including statistical analysis, leads to the conclusion that patients receiving histamine plus IL-2 generally will experience a similar QoL compared to patients receiving IL-2 alone. In other words, histamine when used in combination with subcutaneous IL-2 does not cause any degradation in the quality of life. This is especially important when considered in light of the home-based administration for both IL-2 and histamine in this study and the intensive-care hospital based dosing required for high-dose intravenous bolus IL-2. Subcutaneous administration of IL-2 may lead to a significant improvement over high dose intravenous administration of IL-2 in tolerance and quality of life.

Treatment with histamine plus IL-2 improves the duration of survival of melanoma patients with liver metastases, with minimal impact to the patient in terms of toxicity or reduced quality-of-life.

4.7 Supportive Phase 2 Study (Study MP-MA-0103)

Study MP-MA-0103 is a multicenter, open-label, single-arm study to evaluate the safety and efficacy of combined immunotherapy with subcutaneous interleukin-2 and histamine dihydrochloride in patients with advanced malignant melanoma. The dose regimen of histamine and IL-2 was identical to that used in the randomized,

controlled study (Study No. M01) and was to serve as a supportive trial to M01. The objectives of the study are twofold:

- Evaluate the clinical efficacy of subcutaneous histamine dihydrochloride given in conjunction with IL-2 in patients with Stage IV malignant melanoma. The primary measures of clinical efficacy are survival and time to progression. Secondary measures of clinical efficacy are objective tumor response rate and duration of response.

- Scientifically evaluate the regulatory role of subcutaneously administered IL-2 and histamine dihydrochloride. T-lymphocyte function will be determined in peripheral blood samples by ζ (zeta) chain expression to measure oxidative stress and caspase 3 activation to measure protection from apoptosis. Monocyte function will be measured by IL-6 and IL-12 production, and granulocyte function will be measured by superoxide generation. Tumor tissue will also be obtained where possible for measurement of ζ chain *in situ* and to measure oxidative stress *in vivo.* The results of the investigations of T-lymphocyte function by ζ chain expression and caspase 3 activation, monocyte function by IL-6 and IL-12 production, and granulocyte function by superoxide generation are not complete at this time.

Study 0103 enrolled patients with advanced malignant melanoma according to inclusion and exclusion criteria that were essentially identical to those used for study M01, except that patients who had failed prior IL-2 therapy were allowed. All 7 of the principal investigators participating in study 0103 also participated in the randomized phase 3 study (M01). An interim survival analysis of study 0103 was performed for the population comprised of all evaluable patients as well as the subset of patients with liver metastases at baseline. The efficacy evaluable population included 35 patients; patients with liver metastases at baseline totaled 10 patients. The data cut-off for analysis was March 8, 2000. The study is ongoing with 123 patients enrolled as of October 31, 2000.

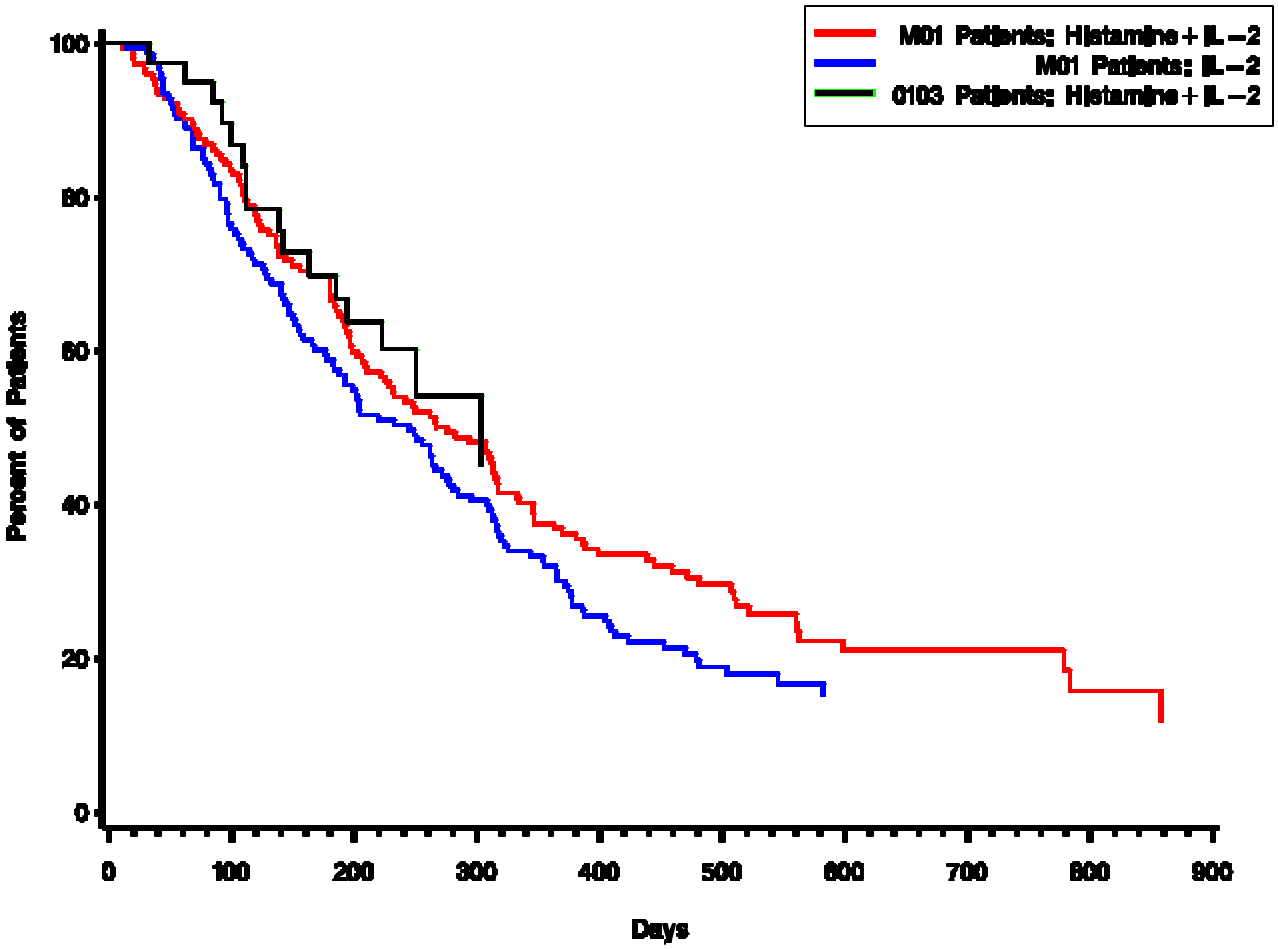
The duration of survival of patients in study 0103 was comparable to the survival of patients in the histamine + IL-2 treatment arm in the Phase 3 randomized study (M01). The interim survival results for study 0103 are summarized and compared to the results for the histamine plus IL-2 treatment arm of study M01 in Table 29. A Kaplan-Meier distribution curve of the survival results from study 0103 is provided in Figure 10, overlaid with the distribution curves from study M01.

Table 29. Median Duration of Survival of Patients in Study 0103 Compared to the Histamine Treatment Arm in Study M01

Population	Study 0103 Histamine + IL-2	Study M01 Histamine + IL-2 Treatment Arm
All Patients with Liver Metastases at Study Entry	249 (126 – 305) (N = 10)	283 (197 – 387) (N = 55)
All Patients	305 (190 -)[a] (N = 35)	272 (211 – 318) (N = 152)

a) 95% confidence interval upper bound cannot be calculated

Figure 10. Proportion of Patients Surviving vs. Time for All Randomized Patients in Study M01 and Study 0103 Histamine + IL-2 vs. IL-2 Alone



4.8 Safety

4.8.1 Extent of Exposure

The safety database for NDA 21-240 is comprised of information on all patients and healthy adult volunteers who received at least one dose of test article through December 10, 1999. The database includes 940 subjects from 12 clinical studies and 7 patients who received Histamine Dihydrochloride Injection under an approved compassionate use scenario; the complete database is briefly described in Table 30.

Table 30. All Clinical Studies of Histamine Dihydrochloride Which Comprise the Safety Database for NDA 21-240 (Through December 10, 1999)

Protocol No.	Phase of Study	Indication	No. of Subjects Receiving Histamine	No. of Subjects Receiving Control Drugs or Therapies
MP-MA-0403	1	General Safety in Healthy Adult Volunteers	17	0
MM-1	1/2	Metastatic Melanoma	10	7
MM-2	1/2		32	0
MP-S01	2		13	0
MP-MA-0102	3		65	79
MP-MA-0103	2		35	0
MP-US-M01	3		151	152
MP-MA-0405	2	Chronic Hepatitis C	129	0
AML-1	2	Acute Myelogenous Leukemia	39	0
MP-MA-0201	3		80	78
MP-S02	2	Renal Cell Carcinoma	7	4
I-318A	2		42	0
Compassionate Use	N/A	Various	7	0
		Total	627	320

Table 31 presents summary exposure information for patients with metastatic melanoma treated with histamine dihydrochloride in studies MP-US-M01 and MP-MA-0103. A total of 185 patients with advanced metastatic melanoma received histamine and IL-2 treatment in the two studies. Total exposure ranged from 1 to 285 days, and total histamine dihydrochloride dose ranged from 2 to 570 mg. One patient in study M01 was enrolled and randomized, but withdrew prior to receiving any study medication, thus the total number of patients is 186.

Table 31. Exposure (Phase 2/3 Malignant Melanoma Studies)

	Histamine plus IL-2 (N = 186) [Studies M01 and 0103]
Total Dose of Histamine (mg)	
Mean (± SD)	115.7 (± 94.0)
Median	82.0
Minimum – Maximum	2 – 570
Total Exposure to Histamine (Days)	
Mean (± SD)	57.8 (± 47.0)
Median	41.0
Minimum - Maximum	1 – 285
Total Duration on Study (Weeks)	
Mean (± SD)	16.3 (± 14.01)
Median	12.1
Minimum - Maximum	0.1 – 84.7

a) All patients for whom exposure information was reported.

4.8.2 Adverse Events

4.8.2.1 Adverse Events in Melanoma Studies with Histamine and IL-2; Incidence by Treatment Group.

Adverse events reported by patients in the melanoma studies with histamine and IL-2 are summarized in this section. A total of 338 patients comprise the safety database of melanoma patients who received treatment with either histamine plus IL-2 (186) or IL-2 alone (152). Table 32 summarizes the demographic characteristics of patients that make up the safety evaluable population of patients receiving histamine plus IL-2 or IL-2 alone. In general the population is mostly male (63.6%), with a median age of 54 years, and is white (97.0%).

Table 32. Summary of Demographics of Safety Population in Metastatic Melanoma Studies

	Histamine plus IL-2 (N = 186) [Studies M01 and 0103]	IL-2 Alone (N = 152) [Study M01]
Gender		
Male	117 (62.9%)	98 (64.5%)
Female	69 (57.5%)	54 (35.5%)
Age		
Mean (± SD)	53.7 (± 13.89)	56.2 (±13.07)
Median	53	56
18-44	51 (27.4%)	26 (17.1%)
45-64	93 (50.0%)	77 (50.7%)
≥ 65	42 (22.6%)	49 (32.2%)
Race		
Caucasian	182 (97.8%)	146 (96.0%)
Black	1 (0.5%)	0
Asian	0	0
Other	3 (1.6%)	6 (3.9%)

Treatment emergent adverse events in melanoma patients are summarized in Table 33 for all patients receiving histamine plus IL-2 and IL-2 alone. All events with an incidence of 5% or more in either group are listed, regardless of relationship to test article.

All patients reported at least one adverse event at some time during their treatment or follow-up period (through 28 days following discontinuation of treatment). This is undoubtedly a reflection of the nature of the underlying disease in addition to the histamine and IL-2 injection therapy.

Table 33. Treatment Emergent Adverse Events
All Treated Patients in Melanoma Studies Receiving Histamine Plus IL-2 or IL-2 Alone

Body System **Preferred Term**	**Histamine plus IL-2 (N = 186) n (%)**		**IL-2 Alone (N = 152) n (%)**		**p-value[a]**
Number of Patients with at Least One Adverse Event	*186*	*(100.0%)*	*152*	*(100.0%)*	
Body as a Whole	*184*	*(98.9%)*	*151*	*(99.3%)*	*0.6845*
Abdomen Enlarged	12	(6.5%)	12	(7.9%)	0.6079
Abdominal Pain	51	(27.4%)	34	(22.4%)	0.2877
Accidental Injury	5	(2.7%)	8	(5.3%)	0.2214
Asthenia	136	(73.1%)	113	(74.3%)	0.7997
Back Pain	27	(14.5%)	23	(15.1%)	0.8742
Chest Pain	45	(24.2%)	25	(16.4%)	0.0809
Chills	137	(73.7%)	110	(72.4%)	0.7910
Face Edema	20	(10.8%)	22	(14.5%)	0.3030
Fever	135	(72.6%)	121	(79.6%)	0.1345
Flu Syndrome	30	(16.1%)	33	(21.7%)	0.1906
Headache	109	(58.6%)	53	(34.9%)	< 0.0001
Infection	23	(12.4%)	12	(7.9%)	0.1802
Injection Site Edema	19	(10.2%)	17	(11.2%)	0.7742
Injection Site Hypersensitivity	26	(14.0%)	15	(9.9%)	0.2503
Injection Site Inflammation	76	(40.9%)	34	(22.4%)	0.0003
Injection Site Mass	48	(25.8%)	41	(27.0%)	0.8088
Injection Site Pain	56	(30.1%)	44	(29.0%)	0.8164
Injection Site Reaction	92	(49.5%)	35	(23.0%)	< 0.0001
Malaise	40	(21.5%)	22	(14.5%)	0.0971
Neck Pain	12	(6.5%)	3	(2.0%)	0.0471
Pain	80	(43.0%)	61	(40.1%)	0.5939
Cardiovascular System	*178*	*(95.7%)*	*104*	*(68.4%)*	*< 0.0001*
Arrhythmia	10	(5.4%)	7	(4.6%)	0.7473
Hypertension	5	(2.7%)	7	(4.6%)	0.3441
Hypotension	91	(48.9%)	30	(19.7%)	< 0.0001
Pallor	13	(7.0%)	12	(7.9%)	0.7520
Palpitation	34	(18.3%)	6	(3.9%)	< 0.0001
Syncope	10	(5.4%)	5	(3.3%)	0.3547
Tachycardia	48	(25.8%)	26	(17.1%)	0.0547
Vasodilation	173	(93.0%)	54	(35.5%)	< 0.0001

a) p-value is by the Cochran-Mantel-Haenszel test comparing Histamine plus IL-2 with IL-2 alone.

Table 33. Treatment Emergent Adverse Events
All Treated Patients in Melanoma Studies Receiving Histamine Plus IL-2 or IL-2 Alone
(continued)

Body System **Preferred Term**	**Histamine plus IL-2 (N = 186) n (%)**		**IL-2 Alone (N = 152) n (%)**		**p-value[a]**
Digestive System	*172*	*(92.5%)*	*145*	*(95.4%)*	*0.2690*
Anorexia	95	(51.1%)	82	(53.9%)	0.5995
Constipation	37	(20.0%)	37	(24.3%)	0.3258
Diarrhea	82	(44.1%)	65	(42.8%)	0.8075
Dry Mouth	25	(13.4%)	16	(10.5%)	0.4149
Dyspepsia	40	(21.5%)	23	(15.1%)	0.1350
Dysphagia	6	(3.2%)	10	(6.6%)	0.1493
Flatulence	9	(4.8%)	11	(7.2%)	0.3533
Nausea	136	(73.1%)	113	(74.3%)	0.7997
Stomatitis	9	(4.8%)	16	(10.5%)	0.0472
Vomiting	114	(61.3%)	90	(59.2%)	0.6978
Hemic and Lymphatic System	*51*	*(27.4%)*	*40*	*(26.3%)*	*0.8203*
Anemia	29	(15.6%)	25	(16.4%)	0.8311
Leukocytosis	7	(3.8%)	7	(4.6%)	0.6996
Metabolic and Nutritional System	*109*	*(58.6%)*	*83*	*(54.6%)*	*0.4612*
Dehydration	16	(8.6%)	12	(7.9%)	0.8147
Edema	20	(10.8%)	9	(5.9%)	0.1151
Peripheral Edema	51	(27.4%)	49	(32.2%)	0.3351
Weight Loss	40	(21.5%)	21	(13.8%)	0.0679
Musculoskeletal System	*92*	*(49.5%)*	*74*	*(48.7%)*	*0.8870*
Arthralgia	32	(17.2%)	35	(23.0%)	0.1823
Bone Pain	18	(9.7%)	8	(5.3%)	0.1303
Myalgia	54	(29.0%)	48	(31.6%)	0.6124
Nervous System	*145*	*(78.0%)*	*111*	*(73.0%)*	*0.2935*
Abnormal Dreams	6	(3.2%)	8	(5.3%)	0.3504
Abnormal Gait	11	(5.9%)	11	(7.2%)	0.6243
Agitation	3	(1.6%)	8	(5.3%)	0.0603
Anxiety	31	(16.7%)	20	(13.2%)	0.3707
Confusion	12	(6.5%)	14	(9.2%)	0.3444
Depression	35	(18.8%)	27	(17.8%)	0.8036
Dizziness	78	(42.0%)	35	(23.0%)	0.0003
Hypesthesia	5	(2.7%)	8	(5.3%)	0.2214
Insomnia	50	(26.9%)	42	(27.6%)	0.8777
Nervousness	16	(8.6%)	7	(4.6%)	0.1472
Paresthesia	22	(11.8%)	7	(4.6%)	0.0185
Somnolence	24	(12.9%)	18	(11.8%)	0.7689
Vertigo	17	(9.1%)	11	(7.2%)	0.5284

a) p-value is by the Cochran-Mantel-Haenszel test comparing Histamine plus IL-2 with IL-2 alone.

Table 33. Treatment Emergent Adverse Events
All Treated Patients in Melanoma Studies Receiving Histamine Plus IL-2 or IL-2 Alone
(continued)

Body System **Preferred Term**	**Histamine plus IL-2** **(N = 186)** **n (%)**		**IL-2 Alone** **(N = 152)** **n (%)**		**p-value[a]**
Respiratory System	*140*	*(75.3%)*	*108*	*(71.1%)*	*0.3837*
Asthma	13	(7.0%)	9	(5.9%)	0.6925
Cough Increased	68	(36.6%)	56	(36.8%)	0.9572
Dyspnea	65	(34.9%)	55	(36.2%)	0.8132
Lung Disorder	16	(8.6%)	12	(7.9%)	0.8147
Pharyngitis	18	(9.7%)	20	(13.2%)	0.3143
Respiratory Disorder	13	(7.0%)	14	(9.2%)	0.4543
Rhinitis	76	(40.9%)	38	(25.0%)	0.0022
Sinusitis	12	(6.5%)	7	(4.6%)	0.4641
Skin and Appendages	*138*	*(74.2%)*	*111*	*(73.0%)*	*0.8088*
Dry Skin	35	(18.8%)	36	(23.7%)	0.2752
Exfoliative Dermatitis	12	(6.5%)	15	(9.9%)	0.2498
Pruritis	56	(30.1%)	25	(16.4%)	0.0035
Rash	51	(27.4%)	46	(30.3%)	0.5659
Skin Disorder	10	(5.4%)	4	(2.6%)	0.2084
Skin Melanoma	25	(13.4%)	23	(15.1%)	0.6583
Sweating	48	(25.8%)	37	(24.3%)	0.7579
Urticaria	10	(5.4%)	5	(3.3%)	0.3547
Special Senses	*74*	*(39.8%)*	*43*	*(28.3%)*	*0.0273*
Conjunctivitis	15	(8.1%)	3	(2.0%)	0.0132
Taste Perversion	34	(18.3%)	19	(12.5%)	0.1466

a) p-value is by the Cochran-Mantel-Haenszel test comparing Histamine plus IL-2 with IL-2 alone.

The Cochran-Mantel-Haenszel method was used to compare the incidence of adverse events between the two groups. While the clinical studies were not designed or powered to detect differences in the incidence of adverse events between the two treatment groups, it is nonetheless instructive to note the differences that were found by observation. Events listed in Table 34 were either significant by the Cochran-Mantel-Haenszel test or occurred at a rate at least double that in the comparator group.

**Table 34. Comparison of Incidence of Adverse Events
All Treated Patients in Melanoma Studies Receiving Histamine Plus IL-2 or IL-2 Alone**

Adverse Events Occurring More Frequently in Patients Receiving Histamine Plus IL-2:	**Histamine plus IL-2**	**IL-2 Alone**
Vasodilation	93.0%	35.5%
Headache	58.6%	34.9%
Injection Site Reaction	49.5%	23.0%
Hypotension	48.9%	19.7%
Dizziness	42.0%	23.0%
Injection Site Inflammation	40.9%	22.4%
Rhinitis	40.9%	25.0%
Pruritis	30.1%	16.4%
Palpitation	18.3%	3.9%
Paresthesia	11.8%	4.6%
Conjunctivitis	8.1%	2.0%
Neck Pain	6.5%	2.0%
Skin Disorder	5.4%	2.6%
Adverse Events Occurring More Frequently in Patients Receiving IL-2 Alone:		
Stomatitis	4.8%	10.5%
Dysphagia	3.2%	6.6%
Agitation	1.6%	5.3%

4.8.2.2 Adverse Events in Melanoma Studies with Histamine and IL-2; Incidence by Severity and Treatment Group.

Treatment emergent adverse events in melanoma patients are summarized in Table 35 by severity for all patients receiving histamine plus IL-2 and IL-2 alone. If a patient had more than one occurrence in the same event category, only the most severe occurrence is counted in Table 42. Investigators were requested to categorize each adverse event as mild, moderate, severe, or life-threatening.

Table 35. Treatment Emergent Adverse Events by Severity
All Treated Patients in Melanoma Studies Receiving Histamine Plus IL-2 or IL-2 Alone

Body System **Preferred Term**	**Histamine plus IL-2 (N = 186) n (%)**								**IL-2 Alone (N = 152) n (%)**							
	Mild		Moderate		Severe		Life-threatening		Mild		Moderate		Severe		Life-threatening	
Number of Patients with at Least One Adverse Event	7	(3.8%)	73	(39.2%)	86	(46.2%)	20	(10.8%)	5	(3.3%)	54	(35.5%)	77	(50.7%)	16	(10.5%)
Body as a Whole	*22*	*(11.8%)*	97	*(52.2%)*	*65*	*(34.9%)*	*0*	*(0.0%)*	*18*	*(11.8%)*	*81*	*(53.3%)*	*48*	*(31.6%)*	*4*	*(2.6%)*
Abdomen Enlarged	10	(5.4%)	2	(1.1%)	0	(0.0%)	0	(0.0%)	5	(3.3%)	6	(3.9%)	0	(0.0%)	1	(0.7%)
Abdominal Pain	22	(11.8%)	24	(12.9%)	5	(2.7%)	0	(0.0%)	10	(6.6%)	11	(7.2%)	13	(8.6%)	0	(0.0%)
Accidental Injury	1	(0.5%)	3	(1.6%)	1	(0.5%)	0	(0.0%)	6	(3.9%)	1	(0.7%)	1	(0.7%)	0	(0.0%)
Asthenia	48	(25.8%)	61	(32.8%)	26	(14.0%)	0	(0.0%)	38	(25.0%)	56	(36.8%)	19	(12.5%)	0	(0.0%)
Back Pain	10	(5.4%)	12	(6.5%)	5	(2.7%)	0	(0.0%)	6	(3.9%)	10	(6.6%)	7	(4.6%)	0	(0.0%)
Chest Pain	24	(12.9%)	15	(8.1%)	6	(3.2%)	0	(0.0%)	16	(10.5%)	5	(3.3%)	4	(2.6%)	0	(0.0%)
Chills	87	(46.8%)	43	(23.1%)	7	(3.8%)	0	(0.0%)	79	(52.0%)	25	(16.4%)	6	(3.9%)	0	(0.0%)
Face Edema	13	(7.0%)	7	(3.8%)	0	(0.0%)	0	(0.0%)	16	(10.5%)	5	(3.3%)	1	(0.7%)	0	(0.0%)
Fever	78	(41.9%)	52	(28.0%)	5	(2.7%)	0	(0.0%)	64	(42.1%)	52	(34.2%)	5	(3.3%)	0	(0.0%)
Flu Syndrome	14	(7.5%)	15	(8.1%)	1	(0.5%)	0	(0.0%)	19	(12.5%)	14	(9.2%)	0	(0.0%)	0	(0.0%)
Headache	69	(37.1%)	25	(13.4%)	15	(8.1%)	0	(0.0%)	40	(26.3%)	10	(6.6%)	3	(2.0%)	0	(0.0%)
Infection	12	(6.5%)	9	(4.8%)	2	(1.1%)	0	(0.0%)	7	(4.6%)	5	(3.3%)	0	(0.0%)	0	(0.0%)
Injection Site Edema	13	(7.0%)	6	(3.2%)	0	(0.0%)	0	(0.0%)	9	(5.9%)	8	(5.3%)	0	(0.0%)	0	(0.0%)
Injection Site Hypersensitivity	18	(9.7%)	8	(4.3%)	0	(0.0%)	0	(0.0%)	10	(6.6%)	5	(3.3%)	0	(0.0%)	0	(0.0%)
Injection Site Inflammation	65	(34.9%)	10	(5.4%)	1	(0.5%)	0	(0.0%)	23	(15.1%)	11	(7.2%)	0	(0.0%)	0	(0.0%)
Injection Site Mass	38	(20.4%)	10	(5.4%)	0	(0.0%)	0	(0.0%)	34	(22.4%)	7	(4.6%)	0	(0.0%)	0	(0.0%)
Injection Site Pain	38	(20.4%)	18	(9.7%)	0	(0.0%)	0	(0.0%)	31	(20.4%)	12	(7.9%)	1	(0.7%)	0	(0.0%)
Injection Site Reaction	74	(39.8%)	18	(9.7%)	0	(0.0%)	0	(0.0%)	24	(15.8%)	11	(7.2%)	0	(0.0%)	0	(0.0%)
Malaise	27	(14.5%)	11	(5.9%)	2	(1.1%)	0	(0.0%)	13	(8.6%)	7	(4.6%)	2	(1.3%)	0	(0.0%)
Neck Pain	8	(4.3%)	4	(2.2%)	0	(0.0%)	0	(0.0%)	2	(1.3%)	0	(0.0%)	1	(0.7%)	0	(0.0%)
Pain	41	(22.0%)	23	(12.4%)	16	(8.6%)	0	(0.0%)	29	(19.1%)	18	(11.8%)	13	(8.6%)	1	(0.7%)

Table 35. Treatment Emergent Adverse Events by Severity
All Treated Patients in Melanoma Studies Receiving Histamine Plus IL-2 or IL-2 Alone (continued)

Body System Preferred Term	Histamine plus IL-2 (N = 186) n (%)								IL-2 Alone (N = 152) n (%)							
	Mild		Moderate		Severe		Life-threatening		Mild		Moderate		Severe		Life-threatening	
Cardiovascular System	*99*	*(53.2%)*	*64*	*(34.4%)*	*11*	*(5.9%)*	*4*	*(2.2%)*	*68*	*(44.71%)*	*24*	*(15.81%)*	*9*	*(5.9%)*	*3*	*(2.0%)*
Arrhythmia	10	(5.4%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	7	(4.6%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
Hypertension	2	(1.1%)	3	(1.6%)	0	(0.0%)	0	(0.0%)	4	(2.6%)	3	(2.0%)	0	(0.0%)	0	(0.0%)
Hypotension	66	(35.5%)	23	(12.4%)	1	(0.6%)	1	(0.6%)	22	(14.5%)	6	(3.9%)	1	(0.7%)	1	(0.7%)
Pallor	11	(5.9%)	2	(1.1%)	0	(0.0%)	0	(0.0%)	12	(7.9%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
Palpitation	31	(16.7%)	3	(1.6%)	0	(0.0%)	0	(0.0%)	6	(3.9%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
Syncope	4	(2.2%)	3	(1.6%)	1	(0.6%)	2	(1.1%)	3	(2.0%)	1	(0.7%)	0	(0.0%)	1	(0.7%)
Tachycardia	36	(19.4%)	10	(5.4%)	2	(1.1%)	0	(0.0%)	21	(13.8%)	5	(3.2%)	0	(0.0%)	0	(0.0%)
Vasodilation	127	(68.3%)	42	(22.6%)	4	(2.2%)	0	(0.0%)	45	(29.6%)	7	(4.6%)	2	(1.3%)	0	(0.0%)
Digestive System	*72*	*(38.7%)*	*70*	*(37.6%)*	*28*	*(15.1%)*	*2*	*(1.1%)*	*49*	*(32.2%)*	*69*	*(45.41%)*	*26*	*(17.1%)*	*1*	*(0.7%)*
Anorexia	60	(32.3%)	31	(16.7%)	4	(2.2%)	0	(0.0%)	50	(32.9%)	24	(15.8%)	8	(5.3%)	0	(0.0%)
Constipation	23	(12.4%)	13	(7.0%)	1	(0.5%)	0	(0.0%)	26	(17.1%)	10	(6.6%)	1	(0.7%)	0	(0.0%)
Diarrhea	56	(30.1%)	23	(12.4%)	2	(1.1%)	0	(0.0%)	46	(30.3%)	15	(9.9%)	4	(2.6%)	0	(0.0%)
Dry Mouth	24	(12.9%)	1	(0.5%)	0	(0.0%)	0	(0.0%)	13	(8.6%)	3	(2.0%)	0	(0.0%)	0	(0.0%)
Dyspepsia	31	(6.7%)	8	(4.3%)	1	(0.5%)	0	(0.0%)	19	(12.5%)	4	(2.6%)	0	(0.0%)	0	(0.0%)
Dysphagia	6	(3.2%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	2	(1.3%)	8	(5.3%)	0	(0.0%)	0	(0.0%)
Flatulence	7	(3.8%)	2	(1.1%)	0	(0.0%)	0	(0.0%)	6	(3.9%)	5	(3.3%)	0	(0.0%)	0	(0.0%)
Nausea	77	(41.4%)	46	(24.7%)	13	(7.0%)	0	(0.0%)	67	(44.1%)	34	(22.4%)	12	(7.9%)	0	(0.0%)
Stomatitis	8	(4.3%)	1	(0.5%)	0	(0.0%)	0	(0.0%)	12	(7.9%)	4	(2.6%)	0	(0.0%)	0	(0.0%)
Vomiting	64	(34.4%)	38	(20.4%)	12	(6.5%)	0	(0.0%)	48	(31.5%)	34	(22.4%)	8	(5.3%)	0	(0.0%)
Hemic and Lymphatic System	*13*	*(7.0%)*	*31*	*(16.7%)*	*7*	*(3.8%)*	*0*	*(0.0%)*	*13*	*(8.5%)*	*12*	*(7.9%)*	*15*	*(9.9%)*	*0*	*(0.0%)*
Anemia	8	(4.3%)	17	(9.1%)	4	(2.2%)	0	(0.0%)	11	(7.2%)	8	(5.3%)	6	(3.9%)	0	(0.0%)
Leukocytosis	2	(1.1%)	4	(2.2%)	1	(0.5%)	0	(0.0%)	2	(1.3%)	2	(1.3%)	3	(2.0%)	0	(0.0%)

Table 35. Treatment Emergent Adverse Events by Severity
All Treated Patients in Melanoma Studies Receiving Histamine Plus IL-2 or IL-2 Alone (continued)

Body System Preferred Term	Histamine plus IL-2 (N = 186) n (%)								IL-2 Alone (N = 152) n (%)							
	Mild		Moderate		Severe		Life-threatening		Mild		Moderate		Severe		Life-threatening	
Metabolic and Nutritional System	*48*	*(25.8%)*	*42*	*(22.6%)*	*18*	*(9.7%)*	*1*	*(0.5%)*	*32*	*(21.1%)*	*33*	*(21.7%)*	*17*	*(11.2%)*	*1*	*(0.7%)*
Dehydration	4	(2.2%)	7	(3.8%)	5	(2.7%)	0	(0.0%)	1	(0.7%)	7	(4.6%)	3	(2.0%)	1	(0.7%)
Edema	12	(6.5%)	6	(3.2%)	2	(1.1%)	0	(0.0%)	6	(3.9%)	1	(0.7%)	2	(1.3%)	0	(0.0%)
Peripheral Edema	29	(15.6%)	16	(8.6%)	6	(3.2%)	0	(0.0%)	21	(13.8%)	16	(10.5%)	12	(7.9%)	0	(0.0%)
Weight Loss	23	(12.4%)	16	(8.6%)	1	(0.5%)	0	(0.0%)	15	(9.9%)	6	(3.9%)	0	(0.0%)	0	(0.0%)
Musculoskeletal System	*53*	*(28.5%)*	*27*	*(14.5%)*	*12*	*(6.5%)*	*0*	*(0.0%)*	*47*	*(30.9%)*	*16*	*(10.5%)*	*11*	*(7.2%)*	*0*	*(0.0%)*
Arthralgia	20	(10.8%)	10	(5.4%)	2	(1.1%)	0	(0.0%)	28	(18.4%)	6	(3.9%)	1	(0.7%)	0	(0.0%)
Bone Pain	5	(2.7%)	8	(4.3%)	5	(2.7%)	0	(0.0%)	4	(2.6%)	2	(1.3%)	2	(1.3%)	0	(0.0%)
Myalgia	37	(19.9%)	12	(6.5%)	4	(2.2%)	0	(0.0%)	36	(23.7%)	5	(3.3%)	7	(4.6%)	0	(0.0%)
Nervous System	*83*	*(44.6%)*	*48*	*(25.8%)*	*12*	*(6.5%)*	*2*	*(1.1%)*	*49*	*(32.2%)*	*42*	*(27.6%)*	*20*	*(13.2%)*	*0*	*(0.0%)*
Abnormal Dreams	6	(3.2%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	6	(3.9%)	2	(1.3%)	0	(0.0%)	0	(0.0%)
Abnormal Gait	9	(4.8%)	2	(1.1%)	0	(0.0%)	0	(0.0%)	7	(4.6%)	4	(2.6%)	0	(0.0%)	0	(0.0%)
Agitation	1	(0.5%)	2	(1.1%)	0	(0.0%)	0	(0.0%)	4	(2.6%)	4	(2.6%)	0	(0.0%)	0	(0.0%)
Anxiety	18	(9.7%)	13	(7.0%)	0	(0.0%)	0	(0.0%)	10	(6.6%)	8	(5.3%)	2	(1.3%)	0	(0.0%)
Confusion	3	(1.6%)	4	(2.2%)	4	(2.2%)	0	(0.0%)	5	(3.3%)	4	(2.6%)	5	(3.3%)	0	(0.0%)
Depression	20	(10.8%)	13	(7.0%)	2	(1.1%)	0	(0.0%)	21	(13.8%)	3	(2.0%)	3	(2.0%)	0	(0.0%)
Dizziness	66	(35.5%)	12	(6.5%)	0	(0.0%)	0	(0.0%)	27	(17.8%)	6	(3.9%)	2	(1.3%)	0	(0.0%)
Hypesthesia	5	(2.7%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	3	(2.0%)	5	(3.3%)	0	(0.0%)	0	(0.0%)
Insomnia	36	(19.4%)	13	(7.0%)	1	(0.5%)	0	(0.0%)	26	(17.1%)	13	(8.6%)	3	(2.0%)	0	(0.0%)
Nervousness	14	(7.5%)	2	(1.1%)	0	(0.0%)	0	(0.0%)	4	(2.6%)	3	(2.0%)	0	(0.0%)	0	(0.0%)
Paresthesia	20	(10.8%)	2	(1.1%)	0	(0.0%)	0	(0.0%)	6	(3.9%)	1	(0.7%)	0	(0.0%)	0	(0.0%)
Somnolence	13	(7.0%)	7	(3.8%)	3	(1.6%)	1	(0.5%)	7	(4.6%)	6	(3.9%)	5	(3.3%)	0	(0.0%)
Vertigo	17	(9.1%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	11	(7.2%)	0	(0.0%)	0	(0.0%)	0	(0.0%)

Table 35. Treatment Emergent Adverse Events by Severity
All Treated Patients in Melanoma Studies Receiving Histamine Plus IL-2 or IL-2 Alone (continued)

Body System Preferred Term	Histamine plus IL-2 (N = 186) n (%)								IL-2 Alone (N = 152) n (%)							
	Mild		Moderate		Severe		Life-threatening		Mild		Moderate		Severe		Life-threatening	
Respiratory System	*74*	*(39.8%)*	*48*	*(25.8%)*	*16*	*(8.6%)*	*2*	*(1.1%)*	*56*	*(36.8%)*	*38*	*(25.0%)*	*12*	*(7.9%)*	2	(1.3%)
Asthma	9	(4.8%)	4	(2.2%)	0	(0.0%)	0	(0.0%)	7	(4.6%)	2	(1.3%)	0	(0.0%)	0	(0.0%)
Cough Increased	50	(26.9%)	15	(8.1%)	3	(1.6%)	0	(0.0%)	45	(29.6%)	10	(6.6%)	1	(0.7%)	0	(0.0%)
Dyspnea	31	(16.7%)	24	(12.9%)	9	(4.8%)	1	(0.5%)	28	(18.4%)	15	(9.9%)	10	(6.6%)	2	(1.3%)
Lung Disorder	14	(7.5%)	2	(1.1%)	0	(0.0%)	0	(0.0%)	9	(5.9%)	2	(1.3%)	1	(0.7%)	0	(0.0%)
Pharyngitis	17	(9.1%)	1	(0.5%)	0	(0.0%)	0	(0.0%)	17	(11.2%)	3	(2.0%)	0	(0.0%)	0	(0.0%)
Respiratory Disorder	12	(6.5%)	1	(0.5%)	0	g185	0	(0.0%)	9	(5.9%)	4	(2.6%)	1	(0.7%)	0	(0.0%)
Rhinitis	64	(34.4%)	11	(5.9%)	1	(0.0%)	0	(0.0%)	33	(21.7%)	5	(3.3%)	0	(0.0%)	0	(0.0%)
Sinusitis	7	(3.8%)	5	(2.7%)	0	(0.5%) (0.0%)	0	(0.0%)	6	(3.9%)	1	(0.7%)	0	(0.0%)	0	(0.0%)
Skin and Appendages	*71*	*(38.2%)*	*37*	*(19.9%)*	*18*	*(9.7%)*	*12*	*(6.5%)*	*57*	*(37.5%)*	*28*	*(18.4%)*	*15*	*(9.9%)*	*11*	*(7.2%)*
Dry Skin	29	(15.6%)	6	(3.2%)	0	(0.0%)	0	(0.0%)	33	(21.7%)	3	(2.0%)	0	(0.0%)	0	(0.0%)
Exfoliative Dermatitis	10	(5.4%)	2	(1.1%)	0	(0.0%)	0	(0.0%)	13	(8.6%)	2	(1.3%)	0	(0.0%)	0	(0.0%)
Pruritis	45	(24.2%)	9	(4.8%)	2	(1.1%)	0	(0.0%)	17	(11.2%)	6	(3.9%)	2	(1.3%)	0	(0.0%)
Rash	40	(21.5%)	10	(5.4%)	1	(0.5%)	0	(0.0%)	33	(21.7%)	12	(7.9%)	1	(0.7%)	0	(0.0%)
Skin Disorder	8	(4.3%)	2	(1.1%)	0	(0.0%)	0	(0.0%)	3	(2.0%)	1	(0.7%)	0	(0.0%)	0	(0.0%)
Skin Melanoma	0	(0.0%)	3	(1.6%)	10	(5.4%)	12	(6.5%)	1	(0.7%)	1	(0.7%)	10	(6.6%)	11	(7.2%)
Sweating	31	(16.7%)	14	(7.5%)	2	(1.1%)	0	(0.0%)	28	(18.4%)	7	(4.6%)	2	(1.3%)	0	(0.0%)
Urticaria	6	(3.2%)	2	(1.1%)	2	(1.1%)	0	(0.0%)	1	(0.7%)	4	(2.6%)	0	(0.0%)	0	(0.0%)
Special Senses	*57*	*(30.6%)*	*14*	*(7.5%)*	*3*	*(1.6%)*	*0*	*(0.0%)*	*35*	*(23.0%)*	*8*	*(5.3%)*	*0*	*(0.0%)*	*0*	*(0.0%)*
Conjunctivitis	11	(5.9%)	4	(2.2%)	0	(0.0%)	0	(0.0%)	1	(0.7%)	2	(1.3%)	0	(0.0%)	0	(0.0%)
Taste Perversion	32	(17.2%)	1	(0.5%)	1	(0.5%)	0	(0.0%)	17	(11.2%)	2	(1.3%)	0	(0.0%)	0	(0.0%)

4.8.2.3 Serious Adverse Events

Adverse events meeting the definition of serious occurred in 59 patients in each treatment group (31.7% of patients in the histamine plus IL-2 group and 38.8% of patients in the IL-2 alone group). Table 36 summarizes the incidence of serious adverse events in both groups. The events are listed without regard to the assessment of causality. The incidence of serious adverse events was not greatly different between the two treatment groups.

Table 36. Serious Adverse Events
All Treated Patients in Melanoma Studies Receiving Histamine Plus IL-2 or IL-2 Alone

Body System **Preferred Term**	**Histamine plus IL-2 (N = 186) n (%)**		**IL-2 Alone (N = 152) n (%)**	
Number of Patients with at Least One Serious Adverse Event	*59*	*(31.7%)*	*59*	*(38.8%)*
Body as a Whole	*11*	*(5.9%)*	*17*	*(11.2%)*
Abdomen Enlarged	0	(0.0%)	1	(0.7%)
Abdominal Pain	0	(0.0%)	4	(2.6%)
Accidental Injury	0	(0.0%)	1	(0.7%)
Allergic Reaction	0	(0.0%)	1	(0.7%)
Ascites	0	(0.0%)	1	(0.7%)
Asthenia	2	(1.1%)	1	(0.7%)
Cellulitis	1	(0.5%)	2	(1.3%)
Chest Pain	2	(1.1%)	2	(1.3%)
Fever	1	(0.5%)	2	(1.3%)
Flu Syndrome	0	(0.0%)	1	(0.7%)
Generalized Edema	1	(0.5%)	0	(0.0%)
Infection	1	(0.5%)	0	(0.0%)
Neck Pain	1	(0.5%)	0	(0.0%)
Overdose[a]	1	(0.5%)a	0	(0.0%)
Pain	1	(0.5%)	1	(0.7%)

a) The patient received an overdose of coumadin, not histamine or IL-2. The principal investigator reported the event as a serious adverse event – unrelated to either study drug.

Table 36. Serious Adverse Events
All Treated Patients in Melanoma Studies Receiving Histamine Plus IL-2 or IL-2 Alone
(continued)

Body System **Preferred Term**	**Histamine plus IL-2 (N = 186) n (%)**		**IL-2 Alone (N = 152) n (%)**	
Cardiovascular System	*10*	*(5.4%)*	*11*	*(7.2%)*
Atrial Fibrillation	0	(0.0%)	1	(0.7%)
Congestive Heart Failure	1	(0.5%)	3	(2.0%)
Deep Thrombophlebitis	2	(1.1%)	2	(1.3%)
Heart Arrest	0	(0.0%)	1	(0.7%)
Hypotension	2	(1.1%)	1	(0.7%)
Intracranial Hemorrhage	0	(0.0%)	1	(0.7%)
Myocardial Infarct	1	(0.5%)	0	(0.0%)
Peripheral Vascular Disorder	1	(0.5%)	0	(0.0%)
Pulmonary Embolus	1	(0.5%)	1	(0.7%)
Subarachnoid Hemorrhage	1	(0.5%)	0	(0.0%)
Syncope	2	(1.1%)	0	(0.0%)
Tachycardia	1	(0.5%)	0	(0.0%)
Thrombophlebitis	0	(0.0%)	1	(0.7%)
Digestive System	*8*	*(4.3%)*	*10*	*(6.6%)*
Cholecystitis	0	(0.0%)	1	(0.7%)
Gastritis	0	(0.0%)	1	(0.7%)
Gastroenteritis	1	(0.5%)	0	(0.0%)
Gastrointestinal Hemorrhage	4	(2.2%)	3	(2.0%)
Hepatic Failure	2	(1.1%)	0	(0.0%)
Intestinal Obstruction	0	(0.0%)	1	(0.7%)
Intestinal Perforation	0	(0.0%)	1	(0.7%)
Pancreas Disorder	0	(0.0%)	1	(0.7%)
Pancreatitis	0	(0.0%)	1	(0.7%)
Rectal Disorder	0	(0.0%)	1	(0.7%)
Vomiting	1	(0.5%)	1	(0.7%)
Hemic and Lymphatic System	*1*	*(0.5%)*	*4*	*(2.6%)*
Anemia	1	(0.5%)	2	(1.3%)
Leukocytosis	0	(0.0%)	2	(1.3%)
Lymphadenopathy	0	(0.0%)	1	(0.7%)
Metabolic and Nutritional System	*4*	*(2.2%)*	*4*	*(2.6%)*
Bilirubinemia	1	(0.5%)	0	(0.0%)
Dehydration	3	(1.6%)	3	(2.0%)
Electrolyte Abnormality	0	(0.0%)	1	(0.7%)
Hypercalcemia	1	(0.5%)	0	(0.0%)
Hyperkalemia	1	(0.5%)	0	(0.0%)

Table 36. Serious Adverse Events
All Treated Patients in Melanoma Studies Receiving Histamine Plus IL-2 or IL-2 Alone
(continued)

Body System **Preferred Term**	**Histamine plus IL-2 (N = 186) n (%)**	**IL-2 Alone (N = 152) n (%)**
Nervous System	*9 (4.8%)*	*4 (2.6%)*
Aphasia	1 (0.5%)	0 (0.0%)
CNS Neoplasm	2 (1.1%)	0 (0.0%)
Confusion	0 (0.0%)	1 (0.7%)
Convulsion	1 (0.5%)	1 (0.7%)
Dizziness	0 (0.0%)	1 (0.7%)
Neuralgia	0 (0.0%)	1 (0.7%)
Neuropathy	2 (1.1%)	0 (0.0%)
Somnolence	3 (1.6%)	1 (0.7%)
Stupor	1 (0.5%)	0 (0.0%)
Respiratory System	*7 (3.8%)*	*6 (3.9%)*
Dyspnea	4 (2.2%)	4 (2.6%)
Hyperventilation	1 (0.5%)	0 (0.0%)
Lung Edema	1 (0.5%)	0 (0.0%)
Pleural Effusion	1 (0.5%)	0 (0.0%)
Pneumonia	0 (0.0%)	1 (0.7%)
Respiratory Disorder	0 (0.0%)	1 (0.7%)
Skin and Appendages	*22 (11.8%)*	*23 (15.1%)*
Skin Melanoma	22 (11.8%)	23 (15.1%)
Urogenital System	*1 (0.5%)*	*0 (0.0%)*
Urinary Tract Disorder	1 (0.5%)	0 (0.0%)

4.9 Safety Conclusion

Although every patient experienced adverse events during the course of the study, analyses of the incidence of adverse events in the two treatment groups fail to demonstrate significant risks associated with the combined treatment in this population with metastatic melanoma. Most AEs were of mild or moderate severity, and the differences in incidence between treatment groups were due primarily to the expected physiological effects of histamine therapy. Patients in the histamine + IL-2 group had higher incidences of AEs affecting the cardiovascular system (hypotension, palpitation, tachycardia, and vasodilation, and the associated nervous system event of dizziness) and those related to injection site reactions. The majority of these events were mild or moderate in severity, considered related to study drug, and did not result in modifications of study drug administration. The incidence of adverse events did not increase over the course of the study, suggesting that histamine is drug that can be tolerated in long-term usage. A total of 63 serious adverse events were reported in the IL-2 group, compared to

54 SAEs in the histamine + IL-2 group. Most SAEs were considered unrelated to study drug (55/63, 87% in the IL-2 group; 40/54, 74% in the histamine + IL-2 group).

The safety data clearly indicates that histamine can be given safely in combination with subcutaneous IL-2 in patients with metastatic melanoma.

5. Benefit/Risk Assessment

The benefit of the use of Histamine Dihydrochloride for Injection in conjunction with interleukin-2 in the treatment of patients with metastatic melanoma is evidenced by the statistically significant increase in the duration of survival for patients with liver metastases. No other therapy has ever established a significant survival effect in metastatic melanoma. In study M01 the increase in the median duration of survival was 4.3 months (an 84% increase) for patients receiving histamine plus IL-2 compared to IL-2 alone. This difference was highly significant ($p = 0.0080$).

In the ITT-LM group receiving histamine plus IL-2, the proportion of patients surviving 12 months (with no patients lost to follow-up) was greater than would be expected based on historical reports in the literature. In the ITT-LM population, 26% of patients receiving histamine plus IL-2 survived for 24 months compared to 7% of patients receiving IL-2 alone. Balch *et al*.[94] reported a one-year survival rate of 8 - 10% for melanoma patients with liver metastases, whereas in study M01 40% (22/55) of patients receiving histamine plus IL-2 survived 12 months.

The increase in the duration of survival obtained as a result of treatment with histamine plus IL-2 is accomplished with a minimal increase in the incidence of adverse events. The profile of adverse events for patients receiving histamine plus IL-2 compared to those for patients receiving IL-2 alone is not greatly different, although for some events the incidence is greater for patients receiving histamine. Vasodilation, headache, injection site reaction, hypotension, dizziness, injection site inflammation, rhinitis, pruritis, palpitation, paresthesia, conjunctivitis, neck pain, and skin disorder all occurred somewhat more frequently in patients in the histamine plus IL-2 treatment arm compared to those receiving IL-2 alone. Adverse events which may be attributable to histamine are generally mild, transient, resolve without treatment, and leave no sequelae.

5.1 High Dose Intravenous IL-2 (Hospital Based Dosing)

Study M01 has demonstrated that histamine plus subcutaneous IL-2 results in a markedly less toxic treatment regimen than the bolus intravenous high dose IL-2 treatment regimen, a regimen which has not been shown in randomized trials to increase survival. While the two regimens have not been studied in a single phase 3, randomized clinical trial design, it is nevertheless useful to compare the incidence of adverse events between the two. Table 37 summarizes the incidence of all grades of adverse events reported in the approved labeling for interleukin-2 compared to those reported in Maxim's Phase 3 study (M01). Ninety-five percent of patients receiving

high-dose intravenous IL-2 experience grade 3, and 35% experience grade 4 adverse events.

Patients receiving high dose intravenous IL-2 must be hospitalized for the duration of the 14 injection sequence on two occasions (usually five days each with a nine day rest in between if patients are able to tolerate the entire sequence). Patients with normal cardiovascular, pulmonary, hepatic, and CNS function may experience serious, life threatening or fatal adverse events with high dose IV interleukin-2. The FDA approved labeling for Proleukin® (aldesleukin) states, "Adverse events with high dose IV interleukin-2 are frequent, often serious, and sometimes fatal." The approved labeling also indicates that the rate of drug-related deaths in 255 patients with metastatic renal cell carcinoma was 4.3% (11/255); in 270 metastatic melanoma patients who received single agent Proleukin® the rate was 2.2% (6/270).

5.2 Lower Dose Subcutaneous IL-2 (Home Care)

In marked contrast, patients receiving lower dose subcutaneous infusions (over 10 to 20 minutes) may remain at home and either receive injections from a home-care health professional or may be trained to inject themselves. In the study M01, all injections of IL-2 and histamine were given at home. The availability of a home based regimen for IL-2 and histamine is an extremely important benefit for patients with life threatening metastatic melanoma.

5.3 Quality-of-Life

Assessment of the quality-of-life data in study M01, including statistical analysis, leads to the conclusion that patients receiving histamine plus subcutaneous IL-2 generally will experience a very similar QoL compared to patients receiving subcutaneous IL-2 alone. In other words, histamine therapy does not lead to any degradation in the quality of life. This finding is especially important when it is considered that the histamine treatment group received 4 subcutaneous injections per day compared to only 2 for the IL-2 alone group. Patients in both study populations receiving histamine plus IL-2 actually experienced statistically significant *increases* in median quality-adjusted survival compared to IL-2 alone ($p = 0.007$ for the ITT population and $p = 0.010$ for the ITT-LM population).

Table 37. Comparison of Adverse Events, Reported in ³ 10% of Patients with Metastatic Cancer (RCC or Melanoma) Receiving Intravenous Bolus Interleukin-2 Compared to Subcutaneous Interleukin-2 Plus Histamine

	High Dose Intravenous IL-2[a] (N = 525)	Subcutaneous IL-2[b] (N = 152)	Subcutaneous Histamine plus IL-2[c] (N = 185)
Body System Event		n (% of Patients)	
Body as a Whole			
Chills	273 (52.0%)	6 (3.9%)	7 (3.8%)
Fever	152 (29.0%)	5 (3.3%)	5 (2.7%)
Malaise	142 (27.0%)	2 (1.3%)	2 (1.1%)
Asthenia	121 (23.0%)	19 (12.5%)	26 (14.0%)
Infection	68 (13.0%)	0 (0.0%)	2 (1.1%)
Pain	63 (12.0%)	14 (9.2%)	16 (8.6%)
Abdominal Pain	58 (11.0%)	13 (8.6%)	5 (2.7%)
Abdomen enlarged	53 (10.0%)	1 (0.7%)	0 (0.0%)
Cardiovascular			
Hypotension	373 (71.0%)	2 (1.3%)	2 (1.1%)
Tachycardia	121 (23.0%)	0 (0.0%)	2 (1.1%)
Vasodilation	68 (13.0%)	2 (1.3%)	4 (2.2%)
Supraventricular tachycardia	63 (12.0%)	0 (0.0%)	0 (0.0%)
Cardiovascular disorder[d]	58 (11.0%)	0 (0.0%)	0 (0.0%)
Arrhythmia	53 (10.0%)	0 (0.0%)	0 (0.0%)
Digestive			
Diarrhea	352 (67.0%)	4 (2.6%)	2 (1.1%)
Vomiting	263 (50.0%)	8 (5.3%)	12 (6.5%)
Nausea	184 (35.0%)	12 (7.9%)	13 (7.0%)
Stomatitis	116 (22.0%)	0 (0.0%)	0 (0.0%)
Anorexia	105 (20.0%)	8 (5.3%)	4 (2.2%)
Nausea and Vomiting	100 (19.0%)	0 (0.0%)	0 (0.0%)
Nervous			
Confusion	179 (34.0%)	5 (3.3%)	4 (2.2%)
Somnolence	116 (22.0%)	5 (3.3%)	4 (2.2%)
Anxiety	63 (12.0%)	2 (1.3%)	0(0.0%)
Dizziness	58 (11.0%)	2 (1.3%)	0 (0.0%)
Hemic and Lymphatic			
Thrombocytopenia	194 (37.0%)	2 (1.3%)	1 (0.6%)
Anemia	152 (29.0%)	6 (3.9%)	4 (2.2%)
Leukopenia	84 (16.0%)	2 (1.3%)	0 (0.0%)

Table 37. Comparison of Grade 3 and 4 Adverse Events, Reported in ³ 1.0% of Patients with Metastatic Cancer (RCC or Melanoma) Receiving Intravenous Bolus Interleukin-2 Compared to Subcutaneous Interleukin-2 Plus Histamine (continued)

	High Dose Intravenous IL-2[a] (N = 525)	Subcutaneous IL-2[b] (N = 152)	Subcutaneous Histamine plus IL-2[c] (N = 185)
Metabolic and Nutritional			
Bilirubinemia	210 (40.0%)	3 (2.0%)	1 (0.6%)
Creatinine increase	173 (33.0%)	0 (0.0%)	0 (0.0%)
Peripheral edema	147 (28.0%)	12 (7.9%)	6 (3.2%)
SGOT increase	121 (23.0%)	0 (0.0%)	1 (0.6%)
Weight gain	84 (16.0%)	0 (0.0%)	0 (0.0%)
Edema	79 (15.0%)	2 (1.3%)	2 (1.1%)
Acidosis	63 (12.0%)	0 (0.0%)	0 (0.0%)
Hypomagnesemia	63 (12.0%)	0 (0.0%)	1 (0.6%)
Hypocalcemia	58 (11.0%)	1 90.7%)	1 (0.6%)
Alk. Phos. Increased	53 (10.0%)	2 (1.3%)	1 (0.6%)
Respiratory			
Dyspnea	226 (43.0%)	12 (7.9%)	10 (5.4%)
Lung Disorder[f]	126 (24.0%)	1 (0.7%)	0 (0.0%)
Respiratory Disorder	58 (11.0%)	1 (0.7%)	0 (0.0%)
Cough Increased	58 (11.0%)	1 (0.7%)	3 (1.6%)
Rhinitis	53 (10.0%)	0 (0.0%)	1 (0.6%)
Skin and Appendages			
Rash	221 (42.0%)	1 (0.7%)	1 (0.6%)
Pruritis	126 (24.0%)	2 (1.3%)	2 (1.1%)
Exfoliative Dermatitis	95 (18.0%)	0 (0.0%)	0 (0.0%)
Urogenital			
Oliguria	331 (63.0%)	*None*	*None*

[a] IL-2: 600,000 IU / kg (0.037 mg / kg) dose administered every 8 hours by a 15-minute IV infusion for a maximum of 14 doses. Following 9 days of rest, the schedule is repeated for another 14 days, for a maximum of 28 doses per course, as tolerated.

[b] IL-2: 9.0 million IU / m^2, BID, by subcutaneous injection on days 1 and 2 of weeks 1 and 3 and 2.0 million IU / m^2, b.i.d., by subcutaneous injection on days 1 through 5 of weeks 2 and 4 followed by 2 weeks of rest.

[c] IL-2: 9.0 million IU / m^2, BID, by subcutaneous injection on days 1 and 2 of weeks 1 and 3 and 2.0 million IU / m^2, b.i.d., by subcutaneous injection on days 1 through 5 of weeks 2 and 4 followed by 2 weeks of rest. Histamine dihydrochloride: 1 mg, BID, by subcutaneous infusion over not less than 10 minutes on days 1 through 5 of weeks 1, 2, 3, and 4 of a 6 week cycle.

[d] Cardiovascular disorder: fluctuations in blood pressure, asymptomatic ECG changes, CHF.

[e] Respiratory disorder: ARDS, CXR infiltrates, unspecified pulmonary changes.

Note: Skin melanoma is progressive disease. This was not recorded as an adverse event in Proleukin studies.

5.4 Conclusions of Benefit/Risk Assessment

The decision to prescribe any particular medical treatment is of course made by the physician. However, patients with terminal cancer frequently decide for themselves, with advice and counsel from physicians and other health care providers, spouse and family, which course of treatment (if any) will be used. The decision is fundamentally a personal one - to be made within the context of the patient's life experiences, values, and existing co-morbidity. Every patient will have a unique set of life experiences, tolerance for pain and discomfort, and emotional and psychological stability in the face of terminal metastatic melanoma and will view the decision within that context. The physician and the manufacturers of the pharmaceuticals that may be prescribed for treatment and/or palliation are responsible for providing accurate and unbiased information regarding potential benefits and likely risks so that patients can make rational and informed decisions.

Within the setting of terminal metastatic melanoma with liver involvement, the benefit/risk relationship for histamine dihydrochloride given in conjunction with subcutaneous interleukin-2 should be considered highly favorable – the benefits clearly outweigh the risks. There is essentially little or no serious additional risk conferred by the subcutaneous administration of 1 mg histamine dihydrochloride, but the benefit of increased duration of survival may be extremely valuable to patients suffering from a terminal malignant disease. Moreover, while the toxicity of IL-2 is substantial, the benefits obtained from receiving subcutaneous injections of IL-2 at home may offer an option other than receiving bolus intravenous injections every 8 hours in two five-day intensive-care hospital stays.

The sponsor believes that the data presented in this application provide compelling evidence demonstrating that histamine is safe and effective when used adjunctively with Interleukin-2 to extend the duration of survival in patients with advanced malignant melanoma having metastases to the liver. Accordingly, the proposed labeling states under INDICATIONS AND USAGE, "Histamine Dihydrochloride for Injection is indicated for adjunctive use with Interleukin-2 (aldesleukin) in the treatment of adult patients with advanced metastatic melanoma that has metastasized to the liver."

6. Update

The cut-off date for the survival analysis for study M01 submitted in NDA 21-240 on July 18, 2000 was March 8, 2000. An update of the survival information through September 8, 2000 is now available and is summarized in Tables 38 and 39. There were no patients lost to follow-up on September 8, 2000 – the survival status of every patient in study M01 was known on September 8, 2000, the point in time when all living patients would have had at least 18 months follow-up. The updated survival data for both studies M01 and 0103, including Kaplan-Meier distribution curves will be submitted to DODP in the near future.

Table 38. Duration of Survival (Days) in Study M01 through September 8, 2000 for the Intent-to-Treat Population

Population	Histamine Plus IL-2	IL-2 Alone
Number of Patients	152	153
Number of Events as of March 8, 2000	117	126
Number of Events as of September 8, 2000	126	138
p-Value Unadjusted Log-Rank Test	0.0610	
p-Value Adjusted by Holm-Šidák Method	0.0610	

Table 39. Duration of Survival (Days) in Study M01 through September 8, 2000 for the Intent-to-Treat Population with Liver Metastases

Population	Histamine Plus IL-2	IL-2 Alone
Number of Patients	55	74
Number of Events as of March 8, 2000	42	69
Number of Events as of September 8, 2000	46	71
p-Value Unadjusted Log-Rank Test	0.0055	
p-Value Adjusted by Holm-Šidák Method	0.0110	

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