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Combined Treatment with Histamine Dihydrochloride, Interleukin-2 and Interferon-α in Patients with Metastatic Melanoma

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Abstract. *Background: Histamine inhibits phagocyte-derived production of reactive oxygen species and improves the anti-tumour efficiency of interleukin-2 (IL-2) and interferon-alpha (IFN-α) in vitro and in tumour-bearing animals. Patients and Methods: In a phase-II study, twenty-seven patients with stage IV melanoma received subcutanous injections of histamine dihydrochloride (histamine) 1.0 mg and IL-2 2.4 MIU/m^2 twice daily (BID) days 1-5 and 8-12. IFN-α 3 MIU once daily was administered throughout a cycle (days 1–28; n=14). Alternatively, bolus doses of IL-2 10 MIU/m^2 BID days 1 and 2 and histamine days 1–28 (n=13) were administered. The aim was to study efficiency (survival and tumour response), toxicity and histamine pharmacokinetics. Results: The median survival time was 11.3 (2.5–45) months. One patient achieved a complete response and 3 patients had partial responses. The compounds were safely self-administered with low toxicity. Plasma histamine concentrations significantly increased after an injection of histamine over 10 minutes (3±1 vs. 63±27 nmol/l). Conclusion: Histamine, IL-2 and IFN-α treatment is safe, well-tolerated and tumour responses were observed. The putative efficiency of histamine as an adjunct to cytokine therapy in metastatic melanoma needs to be confirmed in larger randomized trials.*

The prognosis for patients with distant metastatic (stage IV) melanoma is poor, with a 5-year survival rate of approximately 6% and a median survival duration of < 8 months (1). High-dose bolus regimens of interleukin-2 (IL-2) have been shown to produce long-term remissions in less than 5% of patients, but with significant toxicity (2). Results from larger randomized trials have not demonstrated any significant survival benefit for regimens with IL-2 as monotherapy or combined either with interferon-alpha (IFN-α) or with chemotherapeutic drugs (3).

In vitro studies have demonstrated that monocyte/macrophage (MO)-derived reactive oxygen species (ROS) inhibit natural killer (NK) cell and T cell functions, including the cell cycle proliferation and activation of antitumour cytotoxicity induced by IL-2 (4, 5). The inhibition is irreversible and eventually leads to NK cell and T cell apoptosis (6, 7). Histamine inhibits the production of ROS in phagocytes and also interacts in a synergistic manner with IL-2 and IFN-α to kill a variety of tumour cells *in vitro* (4). Treatment of tumour-bearing rodents with histamine improves the antitumour efficacy of IL-2 in mice with malignant melanoma and lymphoma (8) and in rats with prostate adenocarcinoma (9) or malignant glioma (10).

In an earlier phase II study combining IL-2 (18 MIU/m^2/d continuous *i.v.* infusion) and IFN-α with or without histamine, we reported that the median survival appeared to be prolonged in melanoma patients receiving histamine/IL-2/ IFN-α compared to IL-2/ IFN-α alone (13.3 *vs*. 6.8 months) (11). The present study was performed to evaluate the toxicity and putative efficiency of lower doses of IL-2, administered together with IFN-α and histamine in an out-patient setting. We also explored histamine pharmacokinetics in plasma and blood during treatment.

Patients and Methods

Inclusion and exclusion criteria. The study was approved by the Ethics Committee at Sahlgrenska University Hospital in Göteborg, Sweden. Informed consent was obtained from each patient prior to the start of treatment.

Inclusion criteria were: male or female between 18 and 75 years of age, histological or cytological evidence of metastatic malignant melanoma and Karnofsky performance status ≥70. Exclusion criteria were: CNS metastases or known seizure disorder, serious cardiovascular disease, asthma, allergy or current gastric ulcer, clinically significant infection, pregnancy, childbearing potential without adequate contraceptive, or breastfeeding. Patients with brain metastases which had not been completely resected at least one month prior to the study were excluded.

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Patients should have a life expectancy of at least 3 months and clinically adequate bone marrow, kidney, cardiac and liver functions. In cases of previous radiation therapy, the indicated lesion(s) in the current study had to be outside the field of radiation, or represent a new lesion appearing in the radiation field. Lesions were evaluated by clinical examination, and tumours with clear circumferences on X-ray, CT or MRI scan were considered measurable. Diffuse hepatomegaly, which could not be measured, was considered not evaluable.

Treatment. Two treatment schedules were allowed. Treatment A: Histamine (Histamine dihydrochloride, Apoteksbolaget, Umea, Sweden) 1.0 mg and IL-2 (Proleukin®, Cetus Corp., Emeryville, CA, USA) 2.4 MIU/m^2 were administered twice daily by subcutaneous injections for two 5-day periods (days 1-5 and 8-12). Three MIU IFN-α (Introna®, Schering-Plough, Stockholm, Sweden), was given once daily *s.c.* from 7 days prior to the administration of histamine and IL-2 as a priming dose and continued daily throughout the treatment cycle (days 1-28; population A). Treatment B: The same as treatment A except that IL-2 10 MIU/m^2 was given as a bolus days 1 and 2 of each 28-day cycle and that histamine was given twice daily on all 28 days of the treatment cycle (population B).

Patients were instructed and properly trained in the self-administration of all the study drugs and they reported to the out-patient clinic or ward for examinations, consultations and laboratory tests. Patients were expected to have complete compliance with the administration of the study drugs and the safety aspects were monitored during the entire treatment period.

Based on the medical status of the patient, individual adjustments of doses and duration of treatment and rest periods could be made by the investigator. Normally histamine was injected over 10 minutes, but if the patient experienced toxicity the injection rate could be increased to 20 minutes, or the doses of IL-2, IFN-α and histamine could be reduced by 50%.

Four maintenance treatment cycles were scheduled. If disease progression was noted with a corresponding decrease in performance status, treatment was permanently discontinued. At complete remission, one further cycle of treatment was planned.

Prior anti-neoplastic therapy with any treatment except for IL-2 was allowed. Concurrent treatment with other anti-malignancy drugs, including corticosteroids, and alternative therapies during the course of the study was not allowed. Omeprazol (Losec®, Hässle, Mölndal, Sweden) was administered as concurrent therapy to reduce the possibility of acid indigestion associated with the administration of histamine.

Tumour assessment. All measurable and non-measurable lesions were recorded at baseline and at subsequent assessments scheduled at approximately 8-week intervals. Assessment of the lesions was made by X-ray, CT scan, MRI, ultrasound and by physical palpation.

Duration of survival. Duration of survival was defined as the time elapsed between the date of the first dose of IFN-α and the time of death by any cause.

Overall tumour response. Overall tumour response was defined as the best score obtained in the sequential estimations of tumour response in each patient. Overall tumour response is classified as one of the following according to the WHO classification: complete remission (CR), partial remission (PR), stable disease (SD), or progressive disease (PD).

Table I. *Demographic summary for population A and B.*

	Population A No. of patients	Population B No. of patients
Patients included	14	13
Males / Females	7 / 7	9 / 4
Mean age years (range)	50 (30-72)	51 (27-69)
Karnofsky status		
100-90%	1	6
80-70%	13	7
Prior chemotherapy	7	2
No. of organ sites		
1	4	6
2	5	3
>2	5	4
AJCC Stage		
M1a	5	0
M1b	2	6
M1c	7	7

AJCC, American Joint Committee on Cancer. M1a; Distant skin, subcutaneous or nodal metastases. M1b; Lung metastases. M1c; All other visceral metastases or any distant metastasis with elevated LDH.

Histamine pharmacokinetics. In 20 patients, blood was drawn repeatedly during one or more treatment cycles for determination of blood or plasma concentrations of histamine. In four patients, samples were drawn pre-dose and 2.5, 5, 7.5, 10, 15, 20, 30, 60, 120, 180, 240 and 300 minutes post initiation of injection. Histamine was analyzed using commercial radioimmunoassay (Immunotech S.A, Marseilles, France) with a detection limit of 0.2 nmol/l and with a cross-reactivity of 0.007% with other histamine metabolites.

For histamine pharmacokinetic (PK) calculation after a *s.c.* injection over 10 minutes, a single compartment, 1st order model was chosen. The basic equation for this type of model is:

$$\text{Concentration (Time)} = ((\text{Dose} * K_{AE} * \text{Time})/(\text{Volume}))\ (e^{-KAE*\text{Time}})$$

where K_{AE}=elimination rate constant.

Model optimization with this data set yielded R^2 values of the order of 0.92. Data points after 120 minutes were eventually removed as they added no value to calculations of PK parameters. A limiting factor was the value at 10 minutes, which contributed to lack of curve fit. With the 10-minute value removed R^2 was 0.96.

Statistics. Data are expressed as mean ± SD, except for in Figures 3 and 4 where mean ±SE are used. Two-sided 95% confidence intervals for median survival time for each of the two dose schedules were calculated. Group comparisons were made using the Mann-Whitney test. A p-value <0.05 was considered significant. The Scientist Version 2.02 software (MicroMath Inc, Salt Lake City, USA) and an associated pharmacokinetic library were used for the PK analysis and Statview (SAS Institute, Cary, USA) was used for other analyses.

Results

Twenty-seven patients were included and treated at the department of Surgery at Sahlgrenska University Hospital. Fourteen patients were recruited into population A and 13 into

Table II. *Survival and overall tumour response.*

	Population A (N = 14)	Population B (N = 13)
Survival* (months)		
Median	15.1	8.0
Mean (± SD)	17.5 (± 10.7)	11.0 (± 8.0)
95% CI	6.5 – 27.0	4.8 – 18.6
Overall response		
CR	1	0
PR	2	1
SD	4	6
PD	7	6

* Time from first dose of study drug to death by any cause

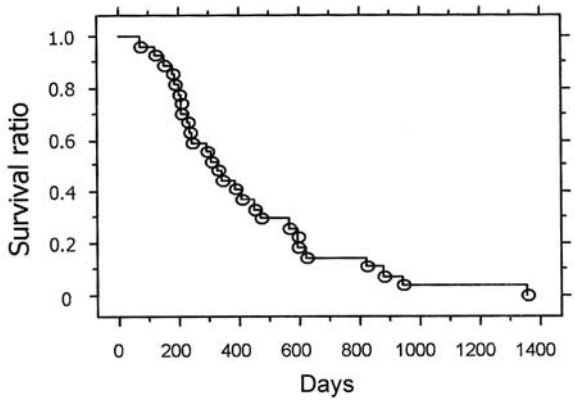

Figure 1. *Kaplan-Meier survival curve for population A and B (n=27).*

population B. The demographic and baseline characteristics of the two populations are summarized in Table I.

The majority of patients in both populations presented with a Karnofsky index of 70-80 upon entry to the protocol. The median duration between metastatic diagnosis and treatment start was similar in populations A and B (4.7 (range 0.4-83) *vs*. 3.3 (range 0.9-37) months). Seven patients in population A and 2 in population B had received prior chemotherapy in addition to surgical intervention. One patient in population A and 2 patients in population B had received prior radiotherapy. No patient had received adjuvant treatment with IFN-α.

Fourteen patients had AJCC (12) stage IV M1c disease and 13 of the 27 patients (48%) had liver metastases at inclusion (7 in population A and 6 in population B). Only 5 patients were stage M1a.

Median survival time from the first dose of any study drug to death was 11.3 (range 2.5-45) months (Table II, Figure 1). There was 1 complete responder (4%), 3 partial responders (11%) and 10 patients with stable disease (37%). Two patients in population A and 1 patient in population B (23% of patients with liver metastases) had an overall partial response with a partial response of liver metastases.

A total of 222 treatment cycles were administered (median 5, range 2-24) to all patients. Six patients received treatment for more than 12 months. The majority of the intended doses of study drugs were administered (% overall exposure in relation to intended dose; IFN-α 83 and 79%, IL-2 78 and 64%, histamine 93 and 95% for populations A and B, respectively). Eleven patients in population A and 11 patients in population B discontinued treatment due to progressive disease. One patient in population A withdrew from treatment for unknown reasons and 2 patients in each population discontinued because of adverse events.

Peripheral blood lymphocytes were counted pretreatment (day-7) and on days 1, 8, 15 and 22. IFN-α pretreatment did not significantly change the lymphocyte count (1.3±0.5 x10^9/l pretreatment and 1.2±0.6 x10^9/l at day 1). After IL-2 treatment, the lymphocyte count increased significantly at day 8 (1.9±0.9 x10^9/l, $p<0.05$) and day 15 (2.2±1.2 x10^9/l, $p<0.01$) *versus* pretreatment. At day 29 (1.3±0.6 x10^9/l), which was the first day of the next cycle, the lymphocyte count was not significantly different from pretreatment.

The adverse events were reported in most cases to be of mild or moderate severity. The adverse events related to IL-2 and IFN-α treatment were, in terms of type and frequency, expected from previous experience. Vasodilatation, fever, chills, asthenia, nausea, headache, metallic taste and anorexia were the most frequently reported adverse events. Grade 3 and 4 adverse events were rare, with asthenia (11%) and nausea (11%) being most frequent. In population A, one patient experienced a myocardial infarction two weeks after receiving the last IL-2 and histamine injections. This patient also experienced angina pectoris during the follow-up period. Another patient in population A, with known angina pectoris, also reported angina pectoris episodes during the treatment cycles. There were no treatment-related deaths during the study (Table III).

Histamine pharmacokinetics. In twenty patients, pretreatment plasma (2.5±1.1 nmol/l) and blood (436±305 nmol/l) histamine concentrations were assayed. Four patients received 1 mg of histamine *via* manual injection over 10 minutes (at 100 μg/min). As described above, the model development resulted in acceptable fits for plasma histamine concentration with R^2=0.96 when the 10-minute time point (63±27 nmol/l) was removed. A maximum concentration (C_{max}) was reached at 9.5 minutes (t_{max}). Half-life of elimination ($t_{1/2}$) was 6.6 min and AUC_{0-t} 1041 nmol-min/l. Simultaneously drawn blood samples showed no change in blood histamine concentrations. Fifteen minutes after the histamine injection (n=46), the plasma histamine concentration increased significantly (17.3±7.8 *vs*. 2.6±1.8 nmol/l before injection, $p<0.0001$; Figure 2) but no significant change in whole blood histamine concentration was observed (427±233 *vs*. 434±196 nmol/l).

Table III. *Incidence of most common adverse events regardless of causality.*

	Population A (N = 14)	Population B (N= 13)	Grade 3 and 4 populations A and B
Asthenia	10 (71%)	9 (69%)	3 (11%)
Chills	13 (93%)	13 (100%)	1 (4%)
Fever	12 (86%)	12 (92%)	1 (4%)
Headache	19 (64%)	8 (62%)	1 (4%)
Vasodilation	14 (100%)	13 (100%)	0
Anemia	4 (29%)	5 (38%)	0
Increased ALAT/ASAT	4 (29%)	4 (31%)	2 (7%)
Angina pectoris	2 (14%)	0	2 (7%)
Hypotension	2 (14%)	1 (8%)	0
Myocardial infarction	1 (7%)	0	1 (4%)
Palpitation	6 (43%)	2 (15%)	0
Syncope	1 (7%)	0	0
Tachycardia	3 (21%)	3 (23%)	0
Anorexia	6 (43%)	8 (62%)	2 (7%)
Diarrhea	9 (64%)	4 (31%)	0
Gastric hemorrhage	4 (29%)	0	2 (7%)
Nausea	8 (57%)	12 (92%)	3 (11%)
Taste perversion	9 (64%)	8 (62%)	0

The histamine concentration during treatment cycles was followed in twenty patients. Compared to pretreatment there was a significant decrease in plasma histamine concentration at day 8 (2.5±1.2 *vs* 1.9±1.3 nmol/l, $p<0.01$; Figure 3A). Blood histamine concentrations were significantly lower at day 1 (348±397 nmol/l) and day 8 (249±190 nmol/l) compared to pretreatment (508±302 nmol/l, $p<0.01$ resp. $p<0.001$; Figure 3B).

The histamine concentration in blood was followed on day 1 for up to 18 cycles. During the first 5 cycles blood samples were collected from 9 to 14 patients, but thereafter 2 to 6 samples was sampled on day 1 in each cycle due to treatment withdrawal. There was no significant change in plasma or blood histamine concentrations from day 1 of treatment cycles 1 through 5. Histamine pharmacokinetics within a cycle and on day 1 of treatment cycles were of similar magnitude in populations A and B.

Discussion

In a previously reported study, patients were treated in hospital with histamine, IFN-α and continuous intravenous IL-2 and the overall survival time and tumour response rate were encouraging (11). The aim of the present study was to design a regimen, which showed clinical efficacy against melanoma and could be safely self-administered by the patient. The three study drugs were given as subcutaneous injections. The original treatment regimen was modified to

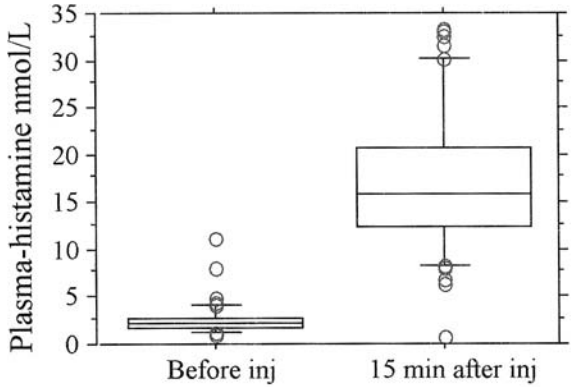

Figure 2. *Plasma-histamine concentration before and 15 minutes after a histamine dihydrochloride injection over 10 minutes. Box & Whiskers plot (n=46).*

evaluate whether daily histamine injections and bolus-dose IL-2 (population B) could be administered with the same aims as for the treatment given to population A. We did not expect any major differences in safety and tumour response between population A and B and the study was not powered to compare the two regimens but to evaluate the combination therapy as part of planning larger randomized trials.

The cytokine doses were calculated to be sufficient to expand and activate the NK cell and T cell populations in peripheral blood with a low frequency of severe grade 3 and 4 toxicity (cardiovascular and neurological). The dose of 1 mg histamine dihydrochloride was considered sufficient to activate and saturate H_2-receptors on phagocytes. *In vitro* data demonstrate that histamine abrogates monocyte-induced suppression of resting and IFN-α-activated NK cells *via* H2-receptors (13). The ED_{50} for histamine response in this latter study was 1 - 2 μM and work by Lanas *et al*. (14) showed that the maximal secretion for gastric mucosa is achieved by an equivalent dose of 2 μM *in vitro*. Christiansen *et al.* demonstrated that histamine doses similar to what was given in our study offered an estimate of the maximal acid secretory capacity (15). Histamine administered systemically (*i.v.*, *s.c.* or inhaled) has been reported to significantly decrease phagocyte function for four hours, with effects lasting up to eight hours, and this effect was mediated by H_2-receptors (16). Taken together, these observations suggest that activation of H2-receptors after systemic administration of histamine may last for four to eight hours and thus probably inhibits the generation of ROS for a similar period of time. Histamine dosing twice daily would then appear appropriate to allow for effective protection of NK cells and T cells.

The treatment regimen was well tolerated as reflected by the number of treatment cycles completed and the duration of treatments. The overall exposure in relation to intended dose was >93% for histamine, demonstrating that dose reduction of histamine was rare. Six patients were treated for more than 12 months and one patient received 24 cycles.

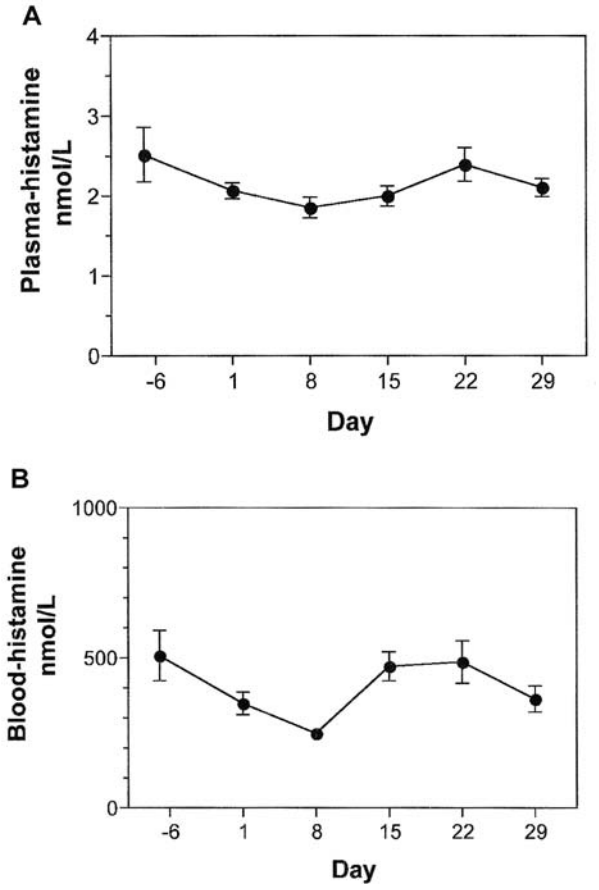

Figure 3. *A. Plasma-histamine concentrations during a treatment cycle. Mean±SE. B. Blood-histamine concentrations during a treatment cycle. Mean±SE.*

Median survival time was 11.3 months in this study. Thirteen out of 27 patients (48%) had liver metastases at study inclusion, 22 patients (81%) had visceral metastases, 20 patients (74%) had a Karnofsky performance status of 70-80 and 17 patients (63%) had 2 or more metastatic sites. Lactic dehydrogenase (LDH) was not routinely taken at the time of this study, but overall the population had a high percentage of poor prognostic factors for expected survival time (12, 17). There was one complete responder (4%), 3 partial responders (11%) and 10 with stable disease (37%) as best overall response in this study. Notably, 3 patients with liver metastases (23%) had an overall partial response with a partial response of their liver metastases.

Previous prospective randomized studies conducted in metastatic melanoma have failed to show a statistically significant increase in survival for any treatment regimen (2, 3, 18-23), including single agent DTIC or temozolomide, combinations of chemotherapeutic drugs, IL-2 as a single drug or in combination with IFN-α, or biochemotherapy. In an analysis of 631 patients treated with various IL-2-based protocols in Europe and the US the median survival was 10.5 months (19), which is within the range of 5-12 months that all of these trials report.

The proposed use of histamine in combination with IL-2 and IFN-α is based on the hypothesis that its mechanism of action would work synergistically with immuno-activating treatment. This is supported by our observation that responding patients to this treatment had additional infiltration of NK cells while patients with progressive disease exhibited a low density of leukocytes infiltrating tumour tissue (24). The results reported here and in our previous study (11) led us to initiate phase III trials. Interestingly, in a randomized study with 305 Stage IV melanoma patients, combined treatment with histamine plus IL-2 significantly improved survival in patients with melanoma liver metastases over IL-2 treatment alone (25). In a two-year update of that study there was a significant survival benefit also in the overall population treated with histamine and IL-2 (26). Enrolment in another randomized phase III study comparing combined treatment with histamine, IL-2 and IFN-α *versus* DTIC has been completed.

The pharmacokinetic parameters of histamine in cancer patients had initially been studied in a few patients in this work. The parameters after an injection over 10 minutes were later confirmed in another study, which also monitored histamine and IL-2 interactions, showing no impact of histamine treatment on the pharmacokinetics of IL-2, with the exception that IL-2 C_{max} and AUC were reduced when histamine was infused 10 minutes after IL-2 (27). The same administrative routine was used in our study. A histamine injection over 10 minutes significantly increased the plasma histamine concentration (from 3 to 63 nmol/l) and the half-life of elimination was 7 minutes. A normalization of plasma histamine concentration was reached within 2 hours. In this study we also measured whole blood histamine concentrations during the injection period without observing any significant changes. This finding was expected since a major part of histamine in whole blood is not free in plasma, but stored in basophilic leukocytes.

To find out whether repeated histamine injections altered histamine metabolism, we monitored plasma and blood histamine concentrations during a cycle and also over several cycles. When compared to pretreatment levels, no significant change in plasma histamine concentration was observed on day 1 of treatment cycles. There was a small but significant decrease in plasma histamine concentration on day 8 and of blood histamine concentrations on days 1 and 8 within a treatment cycle. The magnitude of these concentration variations was small and probably of no clinical relevance. In a few patients histamine metabolites were measured in urine but in no case were the concentrations outside normal reference limits (data not shown).

In summary, this study showed that histamine , IL-2 and IFN-α can be self-administered on an out-patient basis with low toxicity and with observed tumour responses. The putative

efficiency of histamine as an adjunct to cytokine therapy in metastatic melanoma is currently being explored in randomized phase III trials.

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References

1 Barth A, Wanek LA and Morton DL: Prognostic factors in 1,521 melanoma patients with distant metastases. J Am Coll Surg *181*: 193-201, 1995.

2 Atkins MB, Lotze MT, Dutcher JP *et al*: High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. J Clin Oncol *17*: 2105-16, 1999.

3 Atkins MB: Interleukin-2: clinical applications. Semin Oncol *29(3 Suppl 7)*: 12-7, 2002.

4 Hellstrand K, Asea A, Dahlgren C and Hermodsson S: Histaminergic regulation of NK cells. Role of monocyte-derived reactive oxygen metabolites. J Immunol *153*: 4940-7, 1994.

5 Kono K, Salazar-Onfray F, Petersson M *et al*: Hydrogen peroxide secreted by tumour-derived macrophages down-modulates signal-transducing zeta molecules and inhibits tumour-specific T cell- and natural killer cell-mediated cytotoxicity. Eur J Immunol *26*: 1308-13, 1996.

6 Hansson M, Asea A, Ersson U, Hermodsson S and Hellstrand K: Induction of apoptosis in NK cells by monocyte-derived reactive oxygen metabolites. J Immunol *156*: 42-7, 1996.

7 Hansson M, Hermodsson S, Brune M *et al*: Histamine protects T cells and natural killer cells against oxidative stress. J Interferon Cytokine Res *19*: 1135-44, 1999.

8 Asea A, Hermodsson S and Hellstrand K: Histaminergic regulation of natural killer cell-mediated clearance of tumour cells in mice. Scand J Immunol *43*: 9-15, 1996.

9 Johansson S, Landstrom M, Hellstrand K and Henriksson R: The response of Dunning R3327 prostatic adenocarcinoma to IL-2, histamine and radiation. Br J Cancer *77*: 1213-9, 1998.

10 Johansson M, Henriksson R, Bergenheim AT and Koskinen LO: Interleukin-2 and histamine in combination inhibit tumour growth and angiogenesis in malignant glioma. Br J Cancer *83*: 826-32, 2000.

11 Hellstrand K, Naredi P, Lindner P *et al*: Histamine in immunotherapy of advanced melanoma: a pilot study. Cancer Immunol Immunother *39*: 416-9, 1994.

12 Balch CM, Buzaid AC, Soong SJ *et al*: Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. J Clin Oncol *19*: 3635-48, 2001.

13 Hellstrand K, Kylefjord H, Asea A and Hermodsson S: Regulation of the natural killer cell response to interferon-alpha by biogenic amines. J Interferon Res *12*: 199-206, 1992.

14 Lanas AI, Anderson JW, Uemura N and Hirschowitz BI: Effects of cholinergic, histaminergic, and peptidergic stimulation on pepsinogen secretion by isolated human peptic cells. Scand J Gastroenterol *29*: 678-83, 1994.

15 Christiansen PM and Rodbro P: Estimation of maximal gastric acid secretory capacity after augmented histamine stimulation. Scand J Gastroenterol *3*: 305-16, 1968.

16 Bury TB, Corhay JL and Radermecker MF: Histamine-induced inhibition of neutrophil chemotaxis and T-lymphocyte proliferation in man. Allergy *47*: 624-9, 1992.

17 Manola J, Atkins M, Ibrahim J and Kirkwood J: Prognostic factors in metastatic melanoma: a pooled analysis of Eastern Cooperative Oncology Group trials. J Clin Oncol *18*: 3782-93, 2000.

18 Chapman PB, Einhorn LH and Meyers ML *et al*: Phase III multicenter randomized trial of the Dartmouth regimen *versus* dacarbazine in patients with metastatic melanoma. J Clin Oncol *17*: 2745-51, 1999.

19 Keilholz U, Conradt C, Legha SS *et al*: Results of interleukin-2-based treatment in advanced melanoma: a case record-based analysis of 631 patients. J Clin Oncol *16*: 2921-9, 1998.

20 Falkson CI, Ibrahim J, Kirkwood JM, Coates AS, Atkins MB and Blum RH: Phase III trial of dacarbazine *versus* dacarbazine with interferon alpha-2b *versus* dacarbazine with tamoxifen *versus* dacarbazine with interferon alpha-2b and tamoxifen in patients with metastatic malignant melanoma: an Eastern Cooperative Oncology Group study. J Clin Oncol *16*: 1743-51, 1998.

21 Middleton MR, Grob JJ, Aaronson N *et al*: Randomized phase III study of temozolomide *versus* dacarbazine in the treatment of patients with advanced metastatic malignant melanoma. J Clin Oncol *18*: 158-66, 2000.

22 Sparano JA, Fisher RI, Sunderland M *et al*: Randomized phase III trial of treatment with high-dose interleukin-2 either alone or in combination with interferon alfa-2a in patients with advanced melanoma. J Clin Oncol *11*: 1969-77, 1993.

23 Eton O, Legha SS, Bedikian AY *et al*: Sequential biochemotherapy *versus* chemotherapy for metastatic melanoma: results from a phase III randomized trial. J Clin Oncol *20*: 2045-52, 2002.

24 Jörkov AD, Donskov F Steiniche T, Ternesten-Bratel A, Naredi P, Hellstrand K and Hokland M: Immune response in blood and tumour tissue in patients with metastatic malignant melanoma atreated with IL-2, IFN alpha and histamine dihydrochloride. Anticancer Res *23*: 537-42, 2003.

25 Agarwala SS, Glaspy J, O'Day SJ *et al*: Results from a randomized phase III study comparing combined treatment with histamine dihydrochloride plus interleukin-2 *versus* interleukin-2 alone in patients with metastatic melanoma. J Clin Oncol *20*: 125-33, 2002.

26 Agarwala SS, Glaspy J, O'Day SJ *et al*: Results from a randomized phase III study comparing combined treatment with histamine dihydrochloride plus interleukin-2 *versus* interleukin-2 alone in patients with metastatic melanoma. Classic papers and current comments. J Clin Oncol *7*: 589-598, 2002.

27 Middleton M, Sarno M, Agarwala SS *et al*: Pharmacokinetics of histamine dihydrochloride in healthy volunteers and cancer patients: implications for combined immunotherapy with interleukin-2. J Clin Pharmacol *42*: 774-81, 2002.

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