Randomized Double-Blind Placebo-Controlled Trial of Bestatin in Patients With Resected Stage I Squamous-Cell Lung Carcinoma

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Background: **Bestatin is a potent aminopeptidase inhibitor that has immunostimulant and antitumor activity. We conducted a prospective randomized, double-blind, placebo-controlled trial to determine whether postoperative adjuvant treatment with bestatin could prolong the survival of patients with completely resected stage I squamous-cell lung carcinoma.** ***Methods:*** **Patients with confirmed, resected stage I squamous-cell lung carcinoma were randomly assigned to receive either bestatin (30 mg) or placebo daily by mouth for 2 years. We assessed whether bestatin treatment was associated with overall survival and 5-year cancer-free survival and assessed its safety. All statistical tests were two-sided.** ***Results:*** **From July 8, 1992, through March 30, 1995, 402 patients were entered in the study, 202 in the bestatin group and 198 in the placebo group. The median follow-up for surviving patients was 76 months (range = 58–92 months). The 5-year overall survival was 81% in the bestatin group and 74% in the placebo group for a difference of 7% (95% confidence interval [CI] = –1.4% to 15.0%). The 5-year cancer-free survival was 71% in the bestatin group and 62% in the placebo group for a difference of 9% (95% CI = –0.7% to 17.8%). Overall survival (*P* = .033, log-rank test) and cancer-free survival (*P* = .017, log-rank test) were statistically significantly different by Kaplan–Meier analysis. Few adverse events were observed in either group.** ***Conclusions:*** **Survival was statistically significantly better for patients with completely resected stage I squamous-cell lung carcinoma who were treated with bestatin as a postoperative adjuvant therapy than for those who received a placebo. This result requires confirmation in other phase III trials. [J Natl Cancer Inst 2003;95:605–10]**

Bestatin {(–)-*N*-[(2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutyryl]-L-leucine} is a dipeptide immunostimulator that is isolated from a culture filtrate of *Streptomyces olivoreticuli* and that enhances the concanavalin A-induced activation of lymphocytes *(1,2).* Bestatin inhibits aminopeptidase N, aminopeptidase B, and leucine aminopeptidase in mammalian cells *(3,4).* Aminopeptidase N is identical to the myeloid differentiation antigen CD13 *(5,6)* and is a ubiquitous, cell-surface zinc aminopeptidase involved in the inhibition of signals mediated by regulatory peptides *(7–11).* Aminopeptidase N/CD13 is involved in tumor cell invasion *(12,13)* and tumor angiogenesis *(14,15)*, and its expression in tumor tissue is associated with a poor prognosis for patients with resected lymph node-positive colon cancer *(16).*

Bestatin has antitumor activity. In *in vitro* studies, bestatin inhibited the invasion of human metastatic tumor cells *(12,13)*

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and induced apoptosis in human non-small-cell lung cancer cell lines *(17)*. In tumor-bearing mice, bestatin inhibited metastases or tumor growth and prolonged survival *(18–20)*. In clinical studies, bestatin prolonged the survival of patients with acute adult nonlymphocytic leukemia who also received chemotherapy *(21,22)* and had an immunomodulatory effect in patients with lymphoma after autologous bone marrow transplantation *(23)*. Although a small, single-institution, randomized clinical trial of bestatin as a postoperative adjuvant treatment in patients with resected non-small-cell lung cancer did not obtain conclusive results, a subset analysis indicated that bestatin could prolong the survival of patients with completely resected stage I squamous-cell lung carcinoma *(24)*. This result prompted us to conduct a prospective, multicenter, randomized, double-blind, placebo-controlled trial of bestatin as a postoperative adjuvant treatment for patients whose stage I squamous-cell carcinoma was completely resected.

Patients and Methods

Study Design

This trial was initiated by the NK421 Lung Cancer Surgery Group, in which 80 institutions in Japan registered on July 8, 1992. The trial was closed on March 31, 2000. Enrollment was terminated on March 30, 1995. The institutional review board or the ethics committee of each institution reviewed and approved the protocol.

Patients who had undergone the complete resection of a pathologically documented stage I (T1–2, N0, M0) squamous-cell carcinoma were eligible for this study. According to the lung cancer staging system used *(25)*, T1 is a primary tumor that measures 3 cm or less in its greatest dimension, with no invasion of a main bronchus or pleural involvement; T2 is a primary tumor that is larger than 3 cm or a primary tumor of any size that involves the visceral pleura or main bronchus or is associated with atelectasis or obstructive pneumonia that extends to the hilar region; and N0 and M0 indicate the absence of lymph node metastasis and distant metastasis, respectively. Other inclusion criteria were as follows: an age of 40–75 years; no anticancer treatment before operation; no previous cancer or synchronous multiple cancers; adequate organ function after operation; an Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1, or 2 *(26)*; no severe postoperative complications such as pneumonia or pyothorax; and written informed consent from all patients or their representatives.

Confirmation of patient eligibility and randomization were performed by telephone at a central site within 28 days after each patient's operation. There were no stratified factors for randomization. The patients were randomly assigned to study groups in blocks of six by a centralized masked-draw system that combined coded numbers with drug allocation. Each block of six numbers was transmitted from the central office to a pharmacist at each institution who was not involved in the care of the trial patients.

No patient, investigator, or other medical or nursing staff member was aware of the treatment assignments until the final analysis of the study. All statistical analyses were also done in a blinded fashion.

One capsule of either the drug (30 mg) or a placebo (vehicle without active drug) was orally administered after breakfast every day for 2 years. Treatment started within 1 week of randomization. No additional treatment, such as chemotherapy, radiotherapy, or any other biologic response modifier, was allowed until a definitive recurrence or the appearance of a second cancer was documented. Toxicity from either bestatin or placebo was graded according to the criteria of the Japan Society of Clinical Oncology, which consist of a minor modification of the World Health Organization criteria *(27)*. Protocol compliance was measured by checking each patient's diary card regarding capsule administration, general condition, and symptoms at each clinical visit.

A follow-up examination was performed every 3 months for the first 2 years after the patient's operation and every 6 months thereafter. The examination included a physical examination, complete blood count, blood chemistry work-up, serum tumor marker screening, and chest radiography. A computed tomography (CT) scan of the thorax and either a CT scan or an echogram of the upper abdomen were required every 6 months for the first 2 years after the patient's operation. A second primary cancer was defined as a new lung lesion that was found by a histologic examination to be other than squamous-cell carcinoma or that was in a location not related to the location of the primary tumor. The primary end point of the study was overall survival, and the secondary end points were cancer-free survival and the safety assessment.

Statistical Analysis

This trial was designed by assuming a 5-year survival rate of 68% in the placebo group and 80% in the bestatin group. The estimated sample size, with an α error of .05 (two-sided test) and a β error of .2 with a 2-year registration period and a 5-year observation period after the final enrollment, was 188 patients in each group *(28)*. As a result, the registration of a total of 400 patients was planned.

In this study, overall survival was defined as the time from operation until death from any cause, and cancer-free survival was defined as the time from operation until the appearance of the first recurrence of cancer or of the second cancer or death from any cause. Statistical analyses were based on the intent-to-treat method. Survival was estimated by the Kaplan–Meier method, and differences in survival were computed with the log-rank test and the generalized Wilcoxon test. Multivariable analyses with the Cox proportional hazard model were used to estimate the simultaneous effects of prognostic factors on survival *(29)*. After confirmation that the data met the assumptions for a proportional hazards analysis, the stepwise selection was used to ensure more parsimonious models. Variables were retained in the model if the associated two-sided *P* values were .10 or less. Differences between the proportions of patient characteristics were estimated by the χ^2 or *U* test. The data were considered statistically significant when the *P* value was .05 or less. All statistical tests were two-sided.

Results

Characteristics of the Patients

Of the 402 patients enrolled in the trial, two patients rescinded their informed consent before the start of treatment (Fig. 1). The patient characteristics of 202 patients and 198 patients who were randomly assigned to receive bestatin and placebo, respectively, are shown in Table 1. There were no statistically significant differences in the baseline characteristics of the pa-

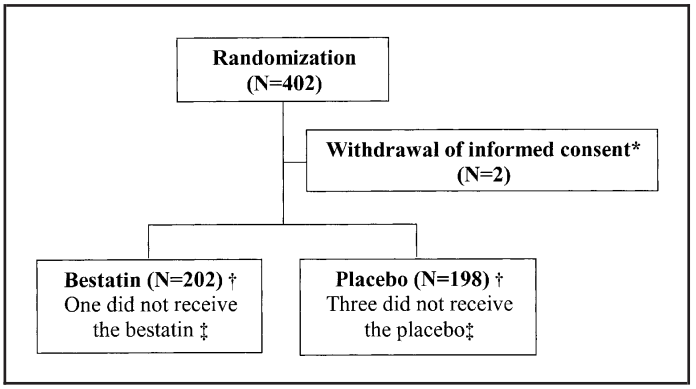

Fig. 1. Trial flow diagram. * = Two patients were excluded from all analyses. † = A total of 400 patients were included in survival analyses. ‡ = Because bestatin or placebo could not be administered (two patients developed empyema, one developed a cerebral infarction, and one moved away), these patients were excluded from the analysis on adverse drug reactions, but they were included in the survival analysis.

Table 1. Patient characteristics

Characteristic	Bestatin group	Placebo group	*P* value* (statistical test)
Total No.	202	198	
Age			
Mean, y	65	66	.183 (*U*)
Range, y	41–76	45–75	
<65 y, No. (%)	83 (41)	66 (33)	.109 (χ^2)
⩾65 y, No. (%)	119 (59)	132 (67)	
Male sex, No. (%)	181 (90)	180 (91)	.660 (χ^2)
ECOG performance status, No. (%)†			.391 (*U*)
0	117 (58)	125 (63)	
1	81 (40)	65 (33)	
2	4 (2)	8 (4)	
Tumor status, No. (%)			.966 (*U*)
Tis‡	0	2 (1)	
1	99 (49)	95 (48)	
2	103 (51)	100 (51)	
3	0	1 (1)	
Location of the tumor, No. (%)			.172 (χ^2)
Rt. upper lobe	44 (22)	64 (32)	
Rt. middle or lower lobe	53 (26)	51 (26)	
Rt. upper and middle lobe§	1 (0)	1 (1)	
Rt. upper and lower lobe§	0	2 (1)	
Lt. upper lobe	60 (30)	45 (23)	
Lt. lower lobe	42 (21)	34 (17)	
Lt. upper and lower lobe§	2 (1)	1 (1)	
Operation modality, No. (%)			.403 (χ^2)
Lobectomy	197 (98)	189 (95)	
Pneumonectomy	5 (2)	8 (4)	
Segmentectomy	0	1 (1)	
Blood transfusion, No. (%)			.970 (χ^2)
Done	36 (18)	35 (18)	
Not done	166 (82)	163 (82)	

*All statistical tests were two-sided.
†ECOG = The Eastern Cooperative Oncology Group; Rt. = right; Lt. = left.
‡Carcinoma *in situ.*
§The tumor was located between the lobes.

tients. Almost all patients underwent a lobectomy, but lobectomy with bronchoplasty was performed in eight patients in the bestatin group and in 18 patients in the placebo group, and lobectomy with either wedge resection or a resection of another lobe was performed in 18 of the patients in the bestatin group and in 11 of the patients in the placebo group. Although nearly all patients had stage I disease, two patients (1%) from the bestatin group and four patients (2%) from the placebo group had a different stage. Stage 0 in two patients from the placebo group was from a carcinoma *in situ.* Stage II from T1N1 (metastasis in the ipsilateral hilar lymph node) M0 disease was observed in two patients from the bestatin group, and stage II from T2N1M0 disease was observed in one patient from the placebo group. Stage IIIA from T3 (involvement of the parietal pleura) N0M0 disease was observed in one patient from the placebo group.

In one of the patients assigned to receive bestatin and three patients assigned to receive placebo, bestatin or placebo could not be administered because two patients developed empyema, one developed a cerebral infarction, and one moved away. These patients were excluded from the analysis on adverse drug reactions, but they were included in the survival analysis.

Adverse Reactions

Table 2 lists the incidence of treatment-related adverse reactions. Data for anorexia were available for 196 patients of the bestatin group and 189 of the placebo group. Few severe adverse reactions were associated with either treatment, and no statistically significant difference in the incidence of adverse reactions (except for anorexia) was observed between the groups. The bestatin group had 18 patients with grade 1 anorexia, six with grade 2 anorexia, and five with grade 3 anorexia; the placebo group had eight with grade 1 anorexia, one with grade 2 anorexia, and four with grade 3 anorexia. The incidence of anorexia of any grade was 15% (29) of the 196 in the bestatin group and 7% (13) of the 189 in the placebo group ($P = .013$).

Twelve patients in the bestatin group discontinued treatment because of adverse reactions after a mean of 162 days (range = 7–644 days), and 11 patients in the placebo group discontinued treatment because of adverse reactions after a mean of 114 days (range = 11–504 days). In total, 97.6 % of the projected dose of bestatin and 96.3 % of the projected dose of placebo were administered.

Overall Survival

The median follow-up for the surviving patients was 76 months (range = 58–92 months). Four patients in the bestatin group were lost to follow-up 330, 768, 1461, and 1535 days after operation, and one in the placebo group was lost to follow-up

Table 2. Treatment-related adverse reactions*

	Bestatin group			Placebo group		
Adverse reaction, % of patients	Grade 1/2	3	4	Grade 1/2	3	4
Leukopenia	<1	0	0	3	<1	0
Thrombocytopenia	2	0	0	<1	0	1
Anemia	2	<1	0	3	<1	0
sGOT†	5	<1	0	4	<1	0
Creatinine	3	0	0	1	<1	<1
Anorexia	12	3	—	5	2	—
Nausea/vomiting	5	2	—	3	2	—
Skin reaction	4	<1	—	9	0	—

*The bestatin group contained 201 patients and the placebo group contained 195 patients. One patient in the bestatin group (n = 202) and three in the placebo group (n = 198) were excluded from the analysis because of no administration of drugs. Grade refers to toxicity grade. — = Grade 4 of anorexia, nausea/vomiting, or skin reaction is not specified.
†sGOT = serum glutamic-oxaloacetic transaminase.

1298 days after operation. These patients were censored on the respective days in the analysis of overall survival. As shown in Fig. 2, the 5-year survival rate was 81% in the bestatin group and 74% in the placebo group for a difference of 7% (95% confidence interval [CI] = –1.4% to 15.0%). Overall survival was statistically significantly different by Kaplan–Meier analysis ($P = .033$ [log-rank test] and $P = .027$ [Wilcoxon test], without a covariate adjustment).

The predetermined covariates were age, sex, ECOG performance status, tumor status, and whether a blood transfusion was received, which are generally observed to affect prognosis *(30,31)*. Because the treatment groups were obligatory, the covariates were selected according to the forward stepwise procedure under the condition that P was less than .1.

The selected covariates (categories compared, and relative risk [RR] with 95% CI; P value) were as follows: age (<65 years versus ⩾65 years, RR = 1.92, 95% CI = 1.24 to 9.95; $P = .003$), performance status (0 versus 1 plus 2, RR = 1.63, 95% CI = 1.13 to 2.35; $P = .010$), blood transfusion (no versus yes, RR = 1.63, 95% CI = 1.08 to 2.47; $P = .021$), and sex (female versus male, RR = 2.28, 95% CI = 1.00 to 5.19; $P = .050$). After adjustment, the treatment group (bestatin versus placebo, RR = 1.49, 95% CI = 1.03 to 2.16; $P = .034$) was almost the same as without any adjustment ($P = .033$).

Pattern of Failure and Cancer-Free Survival

As shown in Table 3, either recurrence or a second primary cancer as the first treatment failure after operation was documented in 29% of the patients in the bestatin group and 37% of those in the placebo group ($P = .066$). The 5-year cancer-free survival was 71% in the bestatin group and 62% in the placebo group for a difference of 9% (95% CI = –0.7% to 17.8%). The 5-year cancer-free survival was statistically significantly different by Kaplan–Meier analysis ($P = .017$ [log-rank test] and $P = .022$ [Wilcoxon test]).

With respect to the initial recurrent site, 27 patients had a local recurrence at the resected margin and/or intrathoracic regional lymph nodes, and 49 patients developed a distant recurrence. Both local and distant recurrences were observed in five patients. Second primary cancers in order of frequency were lung cancer in 15 patients, gastric cancer in nine, hepatoma in six, colon cancer in five, esophageal cancer in three, laryngeal cancer in two, prostate cancer in two, renal cancer in two, bladder cancer in two, and other cancers in three. Twenty-three (40%) of 58 patients with either a recurrence or second primary cancer in the bestatin group underwent a surgical resection, but 40 (54%) of 74 such patients in the placebo group had this procedure. The survival of patients after a diagnosis of either recurrence or second primary cancer was similar for both groups: the 1- and 2-year survival rates after the diagnosis were 50% (95% CI = 37% to 64%) and 37% (95% CI = 24% to 50%) in the bestatin group and 65% (95% CI = 53% to 76%) and 44% (95% CI = 32% to 56%) in the placebo group, respectively ($P = .472$ [log-rank test] and $P = .714$ [Wilcoxon test]).

Table 3. Pattern of treatment failure*

Pattern	Bestatin group	Placebo group
Intrathoracic only, No.		
Local recurrence	12	15
Pulmonary metastases	12	7
Local recurrence plus pulmonary metastases	1	1
Second cancer	5	13
Extrathoracic only, No.		
Recurrence	11	16
Second cancer	14	17
Intrathoracic plus extrathoracic recurrence, No.	3†	3‡
Site nonspecific recurrence, No.	0	2
Total No. (% of all patients)	58 (29)	74 (37)

*The bestatin group contained 202 patients and the placebo group contained 198 patients.

†Intrathoracic recurrence in three patients was pulmonary metastases.

‡Intrathoracic recurrence in three patients was observed in the mediastinal lymph nodes.

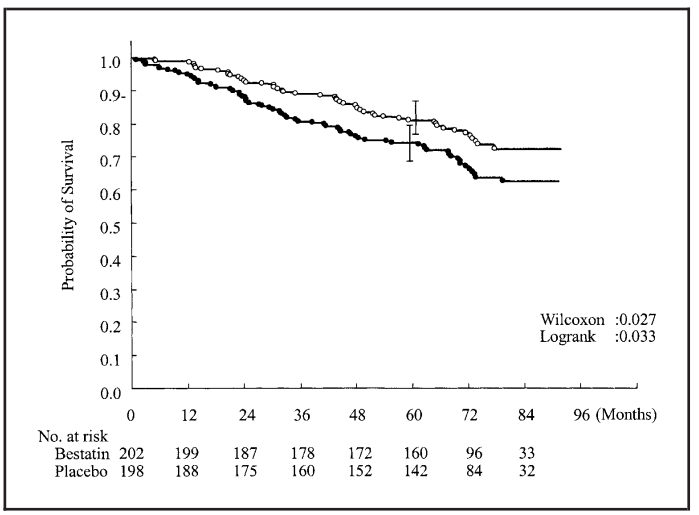

Fig. 2. Overall survival of all 400 patients assigned to the bestatin group (**open circles**) and the placebo (control) group (**solid circles**). Error bars = 95% confidence intervals; $P = .027$ (Wilcoxon test) and $P = .033$ (log-rank test). All statistical tests were two-sided.

DISCUSSION

The events leading to the largest phase III trial for adjuvant treatment in patients with resected stage I non-small-cell lung cancer conducted by the Lung Cancer Study Group from August 30, 1977, to October 20, 1980 *(32),* are worthy of comment. In 1976, McKneally et al. *(33)* reported the results from a randomized trial that compared the survival of resected lung cancer patients who received either a single intrapleural injection of bacillus Calmette-Guérin (BCG) or no further treatment. From a subset analysis of 39 stage I patients in that study, it was found that the survival of patients treated with BCG was better than that of patients who received no further treatment. To verify the results of that trial, the Lung Cancer Study Group performed a randomized, double-blind comparison of 473 patients with resected stage I non-small-cell lung cancer who received either a postoperative intrapleural injection of BCG or a saline solution as a placebo control *(32).* At the time of that study, stage I included T1N0, T2N0, and T1N1M0 disease. The results showed no evidence of improved survival or an extended time to recurrence among the patients given BCG, in contrast to previous findings.

The events leading to the phase III trial described in this article were similar to those for the BCG trial. When we designed the protocol, bestatin had been shown to be an immunostimulator, as had BCG. In 1990, Yasumitsu et al. *(24)* reported

the results of a randomized trial that compared a postoperative bestatin treatment with no treatment in patients with resected non-small-cell lung cancer; a subset analysis of patients with completely resected stage I squamous-cell carcinoma indicated that the 25 patients in the bestatin group had statistically significantly longer survival than the 20 patients in the control group *(24).* Thus on July 8, 1992, we initiated a multicenter, randomized, double-blind, placebo-controlled trial of bestatin as postoperative adjuvant treatment in 402 stage I patients whose squamous-cell lung carcinoma was completely resected. Because the stage and histology were restricted to stage I of T1–2N0 and squamous-cell carcinoma, respectively, the subject population of the present study was more homogenous than that of any previous study, and the sample size was the largest for this group of patients.

Patients treated with bestatin had statistically significantly better survival than patients treated with placebo. Patient characteristics of the bestatin and placebo-controlled groups were well balanced. Although the survival of patients in the bestatin group was better than that of patients in the placebo group, the survival of patients in the placebo group was better than that previously reported *(24,32,33).* In a recent study, the 5-year survival rate of patients with resected stage I squamous-cell carcinoma ranged from 71% to 75% *(34–36),* with survival being defined from operation until lung cancer-related death. Because approximately 30% of patients with resected early-stage lung cancer who died were cancer-free at death *(37),* the 5-year survival rate of 74% in our placebo group in which mortality was defined as death from any cause, thus, appears to be quite high.

Unlike other immunostimulators, including BCG, bestatin has a well defined chemical structure with a molecular weight of 308 and is absorbed well through the small intestine *(1,38).* Bestatin inhibits aminopeptidase N/CD13, which is involved in the angiogenesis of tumors *(14,15)* and invasion of tumor cells *(12,13).* Bestatin induces apoptosis in cancer cells *in vitro (17,39).* In the tumor and on the tumor field, bestatin suppresses metastasis and tumor growth in experimental animal models *(14,18–20).* Bestatin, like other nonspecific immunostimulators, enhances the activity of T lymphocytes and monocytes *(2,18,19).* Aminopeptidase N/CD13 is involved in the presentation of antigens on dendritic cells *(40),* and thus bestatin may play a role in specific immune responses against tumors or microorganisms. These comprehensive effects of bestatin may have contributed to the positive results of our randomized phase III study.

A result of a National Cancer Institute (NCI) Intergroup phase III, placebo-controlled trial to investigate the effect of retinoid isotretinoin in preventing a second primary cancer in 1166 patients with resected stage I non-small-cell lung cancer has been reported *(41).* In that trial, oral administration of retinoid isotretinoin for 3 years did not appear to prevent the development of second primary cancers. In animal studies, the incidence of spontaneous or chemically induced tumors was statistically significantly reduced by the administration of bestatin compared with no administration *(42,43).* Although the aim of the present trial was not to clarify the preventative effect of bestatin on the development of a second primary cancer, the number of patients with second primary cancers as postoperative first treatment failure in the bestatin group appeared to be smaller than that in the placebo group (19 versus 30; $P = .080$). However, some second primary cancers clinically diagnosed were not verified histologically (four in the bestatin group and five in the placebo group) in contrast to those in the NCI Intergroup trial. Therefore, a portion of the second primary cancers observed in our trial might include recurrences, but treatment for either second primary cancer or recurrence did not influence the overall survival of patients in the bestatin and the placebo groups.

Bestatin is currently available in Japan and three other countries as a maintenance treatment for patients with acute nonlymphocytic leukemia who are in complete remission after chemotherapy, as a result of randomized trials for such patients *(21,22).* So far, safety assessments of 2164 patients indicate that bestatin is a safe drug. Adverse reactions are mild and infrequent: the incidence of toxicity of any grade is 1.8% for hepatic dysfunction, 1.3% for skin reaction, and 0.9% for gastrointestinal toxicity including nausea, vomiting, and anorexia.

In conclusion, the oral administration of bestatin as a postoperative adjuvant treatment was associated with a statistically significantly prolonged survival of patients with completely resected stage I squamous-cell carcinoma, without adverse toxic events. However, other phase III trials should be conducted to confirm our conclusions.

APPENDIX

The following principal investigators and institutions also participated in this study:

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