Jpn. J. Cancer Res. 84, 976–981, September 1993

# Follow-up Study of Patients Treated with Monoclonal Antibody-Drug Conjugate: Report of 77 Cases with Colorectal Cancer

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**A total of 77 patients with advanced colorectal cancer, including postoperative patients with liver, lung and peritoneal metastases, were treated with single or multiple injections of monoclonal antibody A7-neocarzinostatin (A7-NCS). A follow-up study of the patients treated with A7-NCS was done and the clinical outcome was compared with that of patients given other chemotherapies. In the postoperative patients with liver metastasis, the A7-NCS treatment prolonged survival time when compared with systemic administration of anticancer drugs, while it showed a similar survival time to chemoembolization using multiple anticancer agents suspended in a lipid contrast medium. Among the patients who underwent surgical resection of primary cancer, with or without liver metastasis, there was no difference in overall 5-year survival rate between the group treated with A7-NCS and the group treated with the other chemotherapies. However, the survival time of the patients treated with A7-NCS was longer than that of the patients treated with the other chemotherapies. In addition, the patients given a higher dose of A7-NCS had a longer survival time than the patients given a lower dose of A7-NCS. Human anti-mouse antibody was detected in all the A7-NCS-treated patients examined. There were no serious side effects in any of the patients given A7-NCS. Thus, this study indicates that the A7-NCS treatment is safe and useful for colorectal cancer patients, though some problems remain, such as optimization of injection dose, route, interval, etc., and overcoming human anti-mouse antibody development.**

Key words: Targeting chemotherapy — Monoclonal antibody — A7-NCS — Colorectal cancer

There has been no follow-up study on a large number of patients treated with monoclonal antibody-drug conjugates, although several promising experimental[1-3] and clinical[4-6] studies have been reported on targeting cancer chemotherapy. Therefore, it is still unknown whether such conjugates will really become a useful modality for cancer patients. We have been engaged in this area of research since 1980 and reported its usefulness for cancer therapy.[7-9] We initiated clinical trials of A7-NCS[2] for colorectal cancer patients in 1985 and obtained some good results such as pain relief, reduction of blood tumor marker and decreased tumor size of liver metastasis.[10] However, the previous study was insufficient to demonstrate the clinical usefulness of A7-NCS, especially whether or not it improves the prognosis of colorectal cancer patients. This prompted us to begin a new study to establish the clinical usefulness of monoclonal antibody-drug conjugates. Our present report is the first article describing the follow-up results in a large number of colorectal cancer patients, treated with a monoclonal antibody-drug conjugate, A7-NCS.

## MATERIALS AND METHODS

**Monoclonal antibody, anticancer drug and its conjugate** The Mab A7 developed by Kotanagi *et al.*[8] is an $IgG_1$ that selectively reacts with human colorectal cancer and recognizes the Mr 45,000 glycoprotein on the cell surface of human colon cancer.[9] NCS, a potent anticancer protein with a high molecular weight of 10,700, was chosen for coupling to Mab A7. NCS[11, 12] is composed of two parts; 1) the chromophore, which is non-proteinous and biologically active, and 2) the apoprotein, which contributes to the stability of the NCS molecule but is irrelevant to the drug activity. NCS was kindly provided by Pola Pharmaceutical Co. Ltd. (Yokohama). The Mab A7 was bound covalently to NCS by the SPDP method as reported previously.[13, 14] One thousand units of NCS was coupled to 15 mg of Mab A7. We have previously shown that A7-NCS has excellent properties *in vitro* and *in vivo* for targeting cancer chemotherapy.[10]

**The patients** The colorectal cancer patients were selected non-randomly. Patients who had undergone hepatectomy due to liver metastasis were excluded from this study.

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[2] Abbreviations: A7-NCS, monoclonal antibody A7-neocarzinostatin conjugate; Mab, monoclonal antibody; SPDP, N-succinimidyl 3-(2-pyridyldithio)propionate; PBS, phosphate-buffered saline; BSA, bovine serum albumin; HAMA, human anti-mouse antibody; 5-FU, 5-fluorouracil; MMC, mitomycin C; ADR, adriamycin.

Table I. Colorectal Cancer Patients Treated with A7-NCS

| Target lesion | No. of patients | Administration route | Dose (units) of A7-NCS as NCS | |
|---|---|---|---|---|
| | | | <4000 | >4000 |
| Primary cases | 55 | i.a. | 26 | 29 |
| Liver metastasis | 21 | i.a. | | |
| Postoperative cases | | | | |
| Liver metastasis | 19 | i.a. | 5 | 14 |
| Lung metastasis | 2 | i.v. | 0 | 2 |
| Peritoneal metastasis | 1 | i.p. | 1 | |

A7-NCS group: Seventy-seven advanced colorectal cancer patients, admitted to our department from 1985 to 1990, were treated with A7-NCS (Table I). They could be subdivided as follows: 1) 55 patients (including 21 with liver metastasis) with advanced colorectal cancer who had undergone surgery for primary tumor removal, 2) 19 patients with multiple liver metastasis who had previously undergone primary tumor resection, 3) two patients who had postoperative multiple lung metastasis, and 4) one patient who had peritoneal metastasis after surgery for colorectal cancer.

Other chemotherapies: Ninety-four colorectal cancer patients, admitted to our department from 1985 to 1990, were treated with other chemotherapies: 1) 59 patients with primary colorectal cancer underwent primary tumor resection and received postoperative conventional chemotherapy such as 5-FU, MMC and ADR; 24 of the 59 patients had liver metastasis and were treated with intra-arterial infusion of 5-FU and MMC; 2) 24 patients with liver metastases, who had previously undergone primary tumor resection, were treated with intra-arterial infusion of aqueous solution of 5-FU and MMC; 3) 11 patients with liver metastases, who had previously undergone primary tumor resection, were treated with intra-arterial chemoembolization[15] using multiple anticancer agents (5-FU, MMC and ADR) suspended in a lipid contrast medium.

**Methods of administration** Thirty-eight patients with primary colorectal cancers and 19 with postoperative liver metastases were given A7-NCS intra-arterially by introducing a catheter inserted from the femoral or subclavian artery to the feeding artery of the tumor. Seventeen patients with primary colon cancers were given the conjugate intra-operatively from an artery proximal to the tumor. Two patients with lung metastases were given A7-NCS intravenously, and one patient with a peritoneal metastasis was given A7-NCS intraperitoneally. For 7 patients with postoperative liver metastases, we surgically placed a subcutaneous infuserport connected to a catheter inserted through the subclavian artery to the hepatic artery for frequent injection of the conjugate. Sixty-three patients were given the conjugate once, and 10 patients with postoperative liver or lung metastases were given it 3 to 5 times on successive days. None of the patients who had undergone surgical resection and A7-NCS treatment received any additional chemotherapy.

**Dosage of A7-NCS** Five patients were injected with 15 mg of A7 and 1,000 units of NCS. Twenty-seven patients were given 30 mg of A7 and 2,000 units of NCS. The other 35 patients received 45 to 90 mg of A7 and 4,000 to 9,000 units of NCS. Six patients with postoperative liver metastases were given intra-arterially 225 mg of A7 and 20,000 units of NCS by 5 consecutive injections from the infuserport.

**HAMA** HAMA of the patients given A7-NCS was examined by ELISA. The details of the procedure were described in a previous report.[16] In brief, the serum samples were taken serially after the A7-NCS injection (Mab A7: 45 mg, NCS: 4,000 units). The patient's serum was added to a 96-well microtiter plate coated with Mab A7 and the plate was incubated for 1 h at room temperature, then washed with PBS containing 1% BSA. Peroxidase-labeled goat anti-human IgG and IgM (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD) were added to each well and the plate was further incubated. After addition of ABTS and 2 $N$ $H_2SO_4$, the absorbance of each well was read with an ELISA reader (Model 2550, Bio-Rad, Richmond, MO).

**Clinical evaluation** Follow-up study was continued after each treatment. Survivals of the colorectal cancer patients treated with A7-NCS and other chemotherapies were compared by dividing the patients into two major groups; one was the patients undergoing a combination of chemotherapy and primary tumor resection and the other was the liver metastatic patients who had previously undergone primary tumor resection. Patients were followed up for 1 to 5 years after the A7-NCS treatment. The cumulative survival curves were generated by the Kaplan-Meiyer method. The difference of survival rates between the patients treated with A7-NCS and other chemotherapies was evaluated by examining the difference of 95% confidence intervals.

## RESULTS

**Survival of patients with post-operative liver metastases** In all the groups, chemotherapeutic agents were administered intraarterially. Fig. 1 shows the survival curves for the patients with liver metastases, treated with intra-arterial injection of A7-NCS, aqueous solution of a mixture of 5-FU and MMC and oil suspension of a mixture of 5-FU, MMC and ADR. There was no difference among the three groups in terms of the status of liver metastasis (volume and number of tumors in the liver at

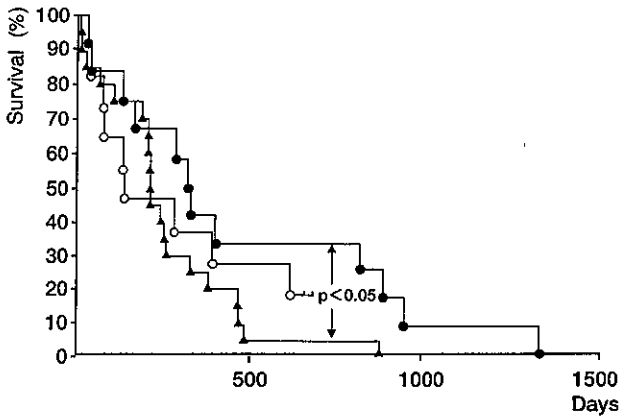


Fig. 1. Survival curves of the 54 patients with postoperative liver metastasis who had previously undergone primary tumor resection. Nineteen, 11 and 24 patients were treated with intra-arterial administration of A7-NCS (●), chemoembolization (○), and conventional chemotherapy (▲), respectively.

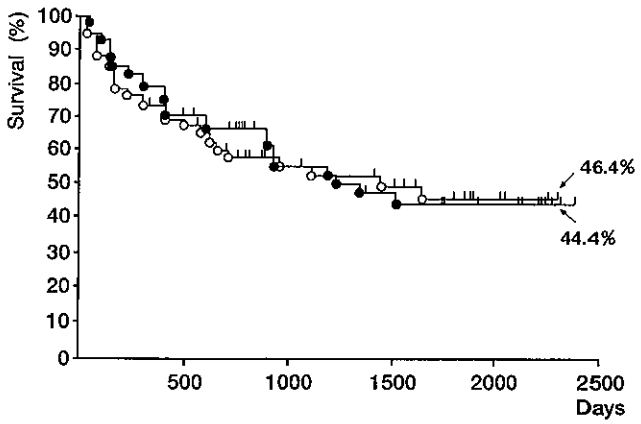


Fig. 2. Overall postoperative 5-year survival of the 114 colorectal cancer patients who underwent primary tumor resection. Fifty-five and 59 patients were treated with A7-NCS (●) and conventional chemotherapy (○), respectively.

Table II. Characteristics of Patients who Underwent Surgery for Colorectal Cancer

| | A7-NCS | Conventional chemotherapy |
|---|---|---|
| Male | 28 | 32 |
| Female | 27 | 27 |
| Age (yr) | 57.8 (34–76) | 60.1 (37–90) |
| TNM | | |
| T0 | 0 | 0 |
| T1 | 1 | 3 |
| T2 | 20 | 25 |
| T3 | 32 | 28 |
| T4 | 2 | 3 |
| NX | 21 | 24 |
| N0 | 18 | 24 |
| N1 | 3 | 2 |
| N2 | 10 | 6 |
| N3 | 3 | 3 |
| M0 | 34 | 35 |
| M1 | 21 | 24 |
| Dukes' | | |
| A | 10 | 10 |
| B | 8 | 14 |
| C | 37 | 35 |

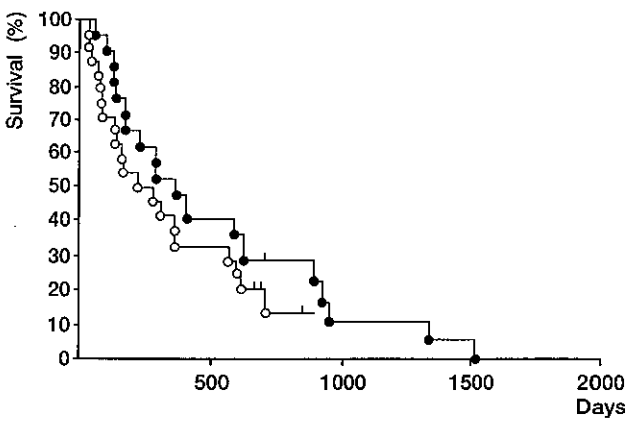


Fig. 3. Survival curves of the 45 colorectal cancer patients with distant metastases who underwent primary tumor resection. Twenty-one and 24 patients were treated with A7-NCS (●) and conventional chemotherapy (○), respectively.

the beginning of administration). The patients given A7-NCS had the highest survival rate. The median survival times were as follows; 328 days for the A7-NCS group, 128 days for the oil suspension group and 230 days for the aqueous solution group. The survival time of the A7-NCS administered group was statistically significantly longer than that of the group treated with aqueous solution of 5-FU and MMC ($P<0.05$). However, there was no significant difference of survival time between the group receiving A7-NCS and the group treated with the oil suspension of 5-FU, MMC and ADR. Two patients with multiple lung metastases and one patient with peritoneal metastasis died of the cancer, showing no response to the conjugate.

**Survival of the patients undergoing chemotherapy and primary tumor resection** Table II shows the characteristics of the patients who underwent resection of colorectal cancer, followed by either the A7-NCS treatment or the

conventional treatment with oral and intravenous administration of 5-FU and MMC. Fig. 2 indicates the overall postoperative survival curves of colorectal cancer patients with and without distant metastasis who underwent tumor resection followed by the A7-NCS treatment or conventional chemotherapy. The 5-year survival rate was 44.4% for the patients given A7-NCS and 46.4% for those given conventional chemotherapy. As shown in Fig. 2, with respect to overall survival rate, there was no difference between these two groups. Fig. 3 shows the postoperative survival curves of the colorectal cancer patients with distant metastases to the liver. There was no difference in the status of liver metastasis between the two groups. The patients given A7-NCS exhibited a slightly higher survival rate than those treated with conventional chemotherapy, but the difference was not statistically significant. In addition, the survival curves of the patients with liver metastasis who were given more than 4,000 units of NCS were compared with those of the patients given less than 4,000 units of A7-NCS. As shown in Fig. 4, the patients given more than 4,000 units of NCS survived longer than those given less than 4,000 units of NCS ($P<0.05$).

**Adverse side effects** There were no serious adverse side effects in the patients receiving the conjugate, regardless of the method of administration (Table III). Of the 77 patients treated with A7-NCS, fever was observed in 35 cases (45.5%) and leucocytosis in 30 cases (38.7%). Other side effects included chest pain, eruption at the site of intracutaneous test injection and slight hypotension with a systolic pressure of about 100 mmHg. These adverse side effects spontaneously disappeared without any treatment.

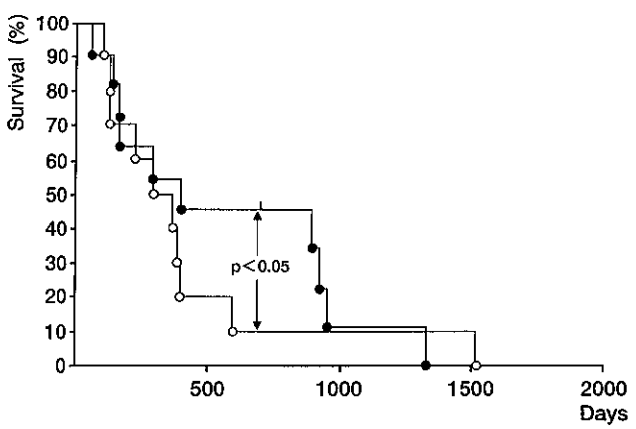


Fig. 4. Survival curves of the 21 patients with liver metastasis who underwent primary tumor resection. Ten and 11 patients were intra-arterially given A7-NCS at more than 4,000 units (●) or less than 4,000 units (○), respectively.

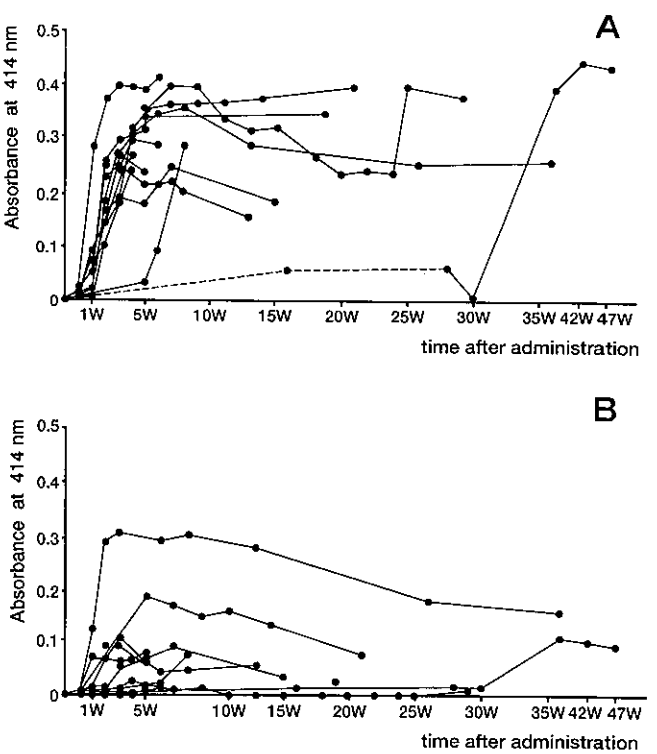


Fig. 5. Time course of IgG class HAMA production (A) and IgM class HAMA production (B).

Table III. Adverse Effects of A7-NCS in 77 Patients

| Adverse effect | No. of patients (%) |
|---|---|
| Fever | 35 (45.5) |
| Leucocytosis more than 10,000 per mm$^3$ | 30 (38.7) |
| Eruption | 2 (2.3) |
| Pain | 2 (2.3) |
| Hypotension | 2 (2.3) |

Table IV. HAMA and Anti-NCS after Injection of A7-NCS

| | No. of patients examined | No. of patients detected |
|---|---|---|
| HAMA | | |
| IgG | 14 | 14 |
| IgM | 14 | 9 |
| IgE | 14 | 0 |
| Anti-NCS antibody | 14 | 0 |

**HAMA** Development of HAMA and anti-NCS in the A7-NCS-administered patients was investigated in 14 patients given A7-NCS. As shown in Table IV, IgG class HAMA was detected in all the patients and IgM class HAMA in 9 patients. IgE class HAMA and anti-NCS antibody were not observed in any of the patients. IgG class HAMA developed from one week after the administration of A7-NCS, and its level reached a peak after 3 to 5 weeks, being maintained for more than 35 weeks (Fig. 5A). The IgM class HAMA was detected at a lower titer and its profile was similar to that of IgG (Fig. 5B).

## DISCUSSION

There has been no information on the clinical outcome of monoclonal antibody-drug conjugate administration to a large number of cancer patients. In this study, we have followed 77 colorectal cancer patients who had received A7-NCS and analyzed the clinical outcomes, such as survival time, adverse side effects and HAMA. In our previous study,[10] A7-NCS was found to produce a tumor reduction on CT scans, reduction of blood tumor marker and also pain relief in colorectal cancer patients with liver metastases. We have now followed these patients to see whether these effects result in improved long-term survival. To evaluate the usefulness of this treatment, it is necessary to conduct a randomized control study with 4 groups, i.e., patients receiving 1) no drug, 2) NCS, 3) non-specific IgG-NCS and 4) A7-NCS. However, we were not able to generate an accurate randomized control for this clinical trial because the treatment is not established as a common drug therapy. Accordingly, A7-NCS was given to a limited number of patients with advanced colorectal cancers. Clinical outcome of A7-NCS treatment was evaluated in the two major groups, each divided into several smaller groups.

First of all, the comparison between A7-NCS treatment and other chemotherapies was performed in those patients with liver metastasis who had previously undergone primary tumor resection. In this trial, no patient survived 5 years after receiving either A7-NCS treatment or other chemotherapy. However, the survival time for the patients treated with A7-NCS was longer than that for the patients treated with other systemic chemotherapies and was almost identical to that of the patients receiving chemoembolization using multiple anticancer agents suspended in a lipid contrast medium (Fig. 1). Although a comparative study of the patients given NCS alone was not performed in this study, the previous reports[17, 18] indicated that NCS alone does not prolong the survival of colorectal cancer patients. Therefore, A7-NCS treatment is superior to both NCS alone and other systemic chemotherapy, and resulted in a longer survival time for patients with liver metastasis of colorectal cancer, although complete cure was not achieved. In another comparison, however, A7-NCS was not superior to chemoembolization, which is the most powerful targeting chemotherapy[15] for liver metastasis currently known. However, the A7-NCS treatment may be better than chemoembolization, if performed at a much higher dose or with a different schedule. The dose of A7-NCS can be increased further and more safely than it can with chemoembolization, since a drug-induced side effect is significantly reduced by Mab conjugation.[19]

In the second trial, the patients underwent A7-NCS treatment or other chemotherapy before or after tumor resection. In this trial, there was no difference between the overall 5-year survival rate of the patients treated with A7-NCS and that of the patients treated with other chemotherapy (Fig. 2). In the patients with distant metastasis, on the other hand, the survival time of the A7-NCS-treated group was longer than that for the groups treated with other chemotherapy (Fig. 3). Furthermore, the patients given a higher dose of A7-NCS had a longer survival time than the group given a lower dose of A7-NCS (Fig. 4). These inconsistent results suggest that the A7-NCS could prolong the survival time of colorectal cancer patients to some degree but could not lead to a complete cure. The reason why there is no difference of survival rate in primary colorectal cancer without distant metastasis can be attributed to the injection dose of A7-NCS, since A7-NCS was administered at a low dose in most of the patients having primary cancer alone. For a complete cure, A7-NCS may require a new design with respect to injection dose, route, time interval, etc.

There were no serious adverse side effects in the patients given the conjugate, regardless of the administration route (Table III). However, HAMA was detected in all the patients examined for the IgG class, in 64% of those examined for the IgM class and none of those examined for the IgE class (Table IV). Anti-NCS antibody was not found at all. The IgG and IgM class HAMAs developed from one week after A7-NCS administration, reached a peak level at 3 to 5 weeks after injection and lasted for more than 35 weeks (Fig. 5A and 5B). These results indicate that immunochemotherapy using murine Mab is limited by the presence of HAMA, when the Mab is injected more than 1 week following the initial injection.[20] HAMA usually increases blood clearance of the Mab administered, reduces Mab tumor accumulation and results in lower therapeutic efficacy of Mab-drug conjugate. In order to overcome this problem, in a new trial we are attempting to inject a sufficient dose of the conjugate into the patients for five successive days while the blood HAMA level is low. Here, despite the administration of a large dose of the conjugate, there is no change of the pharmacokinetics of Mab and no adverse side effects during therapy (data not shown).

Therefore, early multiple injections with a large dose of the conjugate might be one of the ways to circumvent HAMA development. The clinical usefulness of this approach will be reported in the near future.

The clinical observations reported here suggest that monoclonal antibody-drug conjugates may become a useful modality for treatment of cancer patients, although further refinement is still necessary.

ACKNOWLEDGMENTS

This work was supported in part by Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture and the Ministry of Health and Welfare of Japan, and by a Grant-in-Aid from the Ministry of Health and Welfare for the Comprehensive 10-Year Strategy for Cancer Control, Japan.

*(Received March 18, 1993/Accepted June 8, 1993)*

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