The prognostic advantage of preoperative intratumoral injection of OK-432 for gastric cancer patients

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Summary To investigate, by a multi-institutional randomized trial, the prognostic significance of the augmentation of tumour-infiltrating lymphocytes (TILs) by preoperative intratumoral injection of OK-432 (OK-432 it), a bacterial biological response modifier, in patients with gastric cancer. The 10-year survival and disease-free survival were examined and analysis of the factors showing survival benefit was performed. 370 patients who had undergone curative resection of gastric cancer were enrolled in this study and followed up for 10 years postoperatively. Patients were randomized into either an OK-432 it group or a control group. Ten Klinishe Einheit (KE) of OK-432 was endoscopically injected at 1 to 2 weeks before the operation in the OK-432 it group. Both groups received the same adjuvant chemoimmunotherapy consisting of a bolus injection of mitomycin C (0.4 mg kg⁻¹ i.v.) and administration of tegafur and OK-432 from postoperative day 14 up to 1 year later. Tegafur (600 mg day⁻¹) was given orally and OK-432 (5 KE/2 weeks) was injected intradermally for a maintenance therapy. The TILs grades in resected tumour specimens and presence of metastasis and metastatic pattern in dissected lymph nodes were examined. Multivariate analysis was performed to determine the efficacy of OK-432 it on prognostic factors. All patients were followed up for 10 years. The overall 5- and 10-year survival rates and disease-free survival rates of the OK-432 it group were not significantly higher than those of the control group. However, OK-432 it significantly increased the 5- and 10-year survival rates of patients with stage IIIA + IIIB, moderate lymph node metastasis (pN2), and positive TILs. OK-432 it was most effective at prolonging the survival of patients who had both positive TILs and lymph node metastasis. The OK-432 it group with positive TILs showed a significant decrease in metastatic lymph node frequency and in the number of lymph node micrometastatic foci when compared to the control group. This study showed that only one time preoperative OK-432 it, particularly when it triggers TILs, is effective for reduction of regional lymph node metastasis. OK-432 it probably acts partly by eliminating micro-metastatic foci in lymph nodes. Preoperative intratumoral injection of OK-432 is technically very easy and has no serious adverse effects, so it is a promising form of neoadjuvant immunotherapy for advanced gastric cancer. © 2001 Cancer Research Campaign

Keywords: intratumoral injection; OK-432 (picibanil); medical lymphadenectomy; preoperative immunotherapy

Previous research has indicated that cancer patients with high grades of tumour-infiltrating lymphocytes (TILs) have a better prognosis for many tumours, including gastric cancer (MacCarty and Mahle 1921, Black et al, 1956; Miwa et al, 1984). A number of recent studies have also demonstrated that tumour-specific cytotoxic T cells (CTLs) are present in TILs from patients with tumours of various types, including melanoma (Itoh et al, 1986; Topalian et al, 1989), renal cancer (Schenidet al, 1993), ovarian cancer (Jonaides et al, 1993), sarcoma (Slovin et al, 1986), squamous cell cancer of the head and neck (Yasumura et al, 1994), and gastric cancer (Ikeda et al, 1996). About 35 years ago, we found an increase of TILs in resected gastric cancer specimens after intratumoral injection of BCG 1 to 2 weeks before operation. Subsequently, we have performed preoperative intratumoral injection of various biological response modifiers (BRMs), such as BCG-CWS, Nocardia rubra-CWS, OK-432, PSK, and IL-2, in gastric cancer patients (Miwa et al, 1984; Moriyasu et al, 1985; Akura et al, 1986). Any of these BRMs injected 1 to 2 weeks before operation trigger an increase of TILs in the resected tumours of two-thirds of the patients. Preliminary study on preoperative intratumoral injection of OK-432 (OK-432 it) was performed, because OK-432 has been a commercially available BRM which has been widely used in Japan for over 20 years. TILs were moderately positive in 40% and markedly positive in 33% of the patients with preoperative OK-432 it compared with only 31% and 0%, respectively, in the control group without OK-432 it (Miwa et al, 1984). It remains of great interest to determine whether TILs induced by OK-432 it can improve the postoperative prognosis of gastric cancer patients.

Although there has been remarkable development of gastroscopy for the early detection of gastric cancer as well as improvement of surgical techniques during the last 3 decades, the 5-year survival rate of advanced gastric cancer patients undergoing curative resection has remained at 20 to 40% for stage III and 5 to 15% for stage IV (The Japanese Gastric Cancer Study Group 1981; Siewert et al, 1993). Nevertheless, the majority of such patients die of a recurrence based on microscopic residual tumours. In order to eradicate those microscopic residual tumours postoperative adjuvant chemotherapy with intravenous mitomycin C (MMC) and oral 5-FU or 5-FU derivatives like tegafur has been routinely performed for advanced gastric cancer patients in Japan for the last 20 years (Inokuchi et al, 1984; Maehara et al, 1991; Nakazato, 1994). Subsequently, systemic nonspecific immunotherapy with OK-432 has been widely combined with adjuvant chemotherapy, because immunotherapy is generally more effective when the residual tumour mass is quite small (Arinaga et al, 1992).
On the basis of such adjuvant chemoimmunotherapy for gastric cancer patients, we conducted a multi-institutional randomized control trial of adjuvant chemoimmunotherapy consisting of MMC, tegafur, intradermal injection of OK-432 with or without preoperative OK-432 it in gastric cancer patients between June 1985 and December 1986.

The present article is the final report. According to the UICC: TNM classification of gastric cancers (4th edition), we found that OK-432 it significantly improved the 10-year survival of gastric cancer patients with stage IIIA+IIB, positive TILs, pN1 lymph node metastasis. In particular, OK-432 it was useful for patients with positive TILs and pN(+) when used in combination with adjuvant chemoimmunotherapy.

MATERIALS AND METHODS

Stratification and randomization of patients

The procedures have been described previously (Tanaka et al, 1994). Briefly, from April 1985 to December 1986, patients with gastric cancer determined endoscopically to be advanced but curatively resectable were randomly enrolled into either the OK-432 it group or the control group after kindly informed consent.

Protocol and postoperative treatment

Patients in the OK-432 it group had endoscopic injection of OK-432 (10 KE 5 ml-1 saline) into approximately 10 sites in the tumour using NM-1K needles (Olympus Co, Japan) at 7 to 14 days before surgery. Patients in the control group were not treated before the operation. All patients received identical postoperative therapy. Some cases with distant metastasis were enrolled for difficulty of pre-operative staging due to micro foci of liver or peritoneum. Excluding age, there were no significant differences between the two groups, and the age difference had no significant influence on survival according to multivariate analysis and adjusted analysis by age. These were no differences in background between the 39 patients in the OK-432 it group and the 32 patients in the control group with stage IIIA+IIB.

OK-432

OK-432 is a penicillin-treated lyophilized powder of the avirulent Su strain of human Group A Streptococcus pyogenes (Chugai Pharmaceutical, Tokyo, Japan). The Klinische Einheit (KE) unit is used to express the strength of the preparation, with 1 KE of OK-432 being equivalent to 0.1 mg of dried streptococcal cells.

Tegafur

Tegafur (1-(2-tetra hydrouranyl)-5-fluorouracil, MW:200) is a prodrug that is slowly metabolized to 5-FU by microsomal cytochrome p450, which is mainly found in the liver, and soluble enzymes. Tegafur has comparable activity with that of 5-FU.

Histopathological examination

To assess treatment efficacy, haematoxylin-eosin stained resected specimens of the main tumour and dissected lymph nodes were examined independently by 3 pathologists, and the mode evaluations was determined.

TILs were graded as absent, mild, moderate, or marked, with absent or mild TILs being classified as negative and moderate or marked TILs as positive. A total of 2829 dissected lymph nodes was examined for metastasis. The pattern of nodal metastasis was classified as minor-solitary, minor-multiple, major-solitary and major-multiple. The classifications of ‘minor’ or ‘major’ was based on the size of metastatic foci, i.e., under or over 0.2 mm in diameter, while ‘solitary’ or ‘multiple’ was based on the number of metastatic foci on each node.

Statistical analysis

The chi-squared test was used to evaluate the clinical characteristics. The survival rate and disease-free survival rate were estimated by the Kaplan-Meier method, and statistical significance was evaluated by the log-rank test. The comparison of histological findings was performed with Student’s t-test. Cox’s proportional hazards regression was used to assess the impact of treatment after adjustment for other characteristics. Statistical analysis, including multivariate analysis, was performed with Statistical Analysis System (SAS) software.

RESULTS

Clinical and pathological characteristics

Table 1 compares the two groups with respect to sex, age, macroscopic stage, lymph node metastasis, lymph node dissection, operative curability and microscopic findings due to postoperative evaluation. Some cases with distant metastasis were enrolled for difficulty of pre-operative staging due to micro foci of liver or peritoneum. Excluding age, there were no significant differences between the two groups, and the age difference had no significant influence on survival according to multivariate analysis and adjusted analysis by age. These were no differences in background between the 39 patients in the OK-432 it group and the 32 patients in the control group with stage IIIA+IIB.

The grade of TILs in the OK-432 it group were 4 cases in absent, 104 cases in mild, and 51 cases in moderate or marked and in the control group, 13, 100 and 38, respectively. There was a significant difference in the grade of TILs between two groups (P = 0.038).

There were no significant differences between two arms in the total doses of adjuvant chemoimmunotherapy. 55% of OK-432 it group and 58% of control group were administrated more than 75% of the planned doses.

Survival and disease-free survival

At the end of December 1996, all subjects had been followed up for 10 years. 187 of the 370 eligible patients had died. 93 out of 187 patients died of cancer or other disease in the OK-432 it group and 94 out of 183 died in the control group. The survival curves for the two groups are shown in Figure 1A. The overall 5-year survival rate was 58.7% for the OK-432 it group and 56.8% for the control group, while the 10-year survival rate was 49.5% and 48.2%, respectively (P = 0.5085). The disease-free survival rate of curatively resected patients was also not different for the two groups throughout the follow-up period (P = 0.2005) (Figure 1B).
10 year survival with preoperative endoscopically OK-432 intratumoral injection

Table 1  Clinical and pathologic characteristics

| Characteristics                  | Enrolled all patients (n = 370) | Histological stage IIIA+IIIB (n = 71) |
|----------------------------------|----------------------------------|--------------------------------------|
|                                  | OK-432 it (n = 187) | Control (n = 183) | Chi square | OK-432 it (n = 39) | Control (n = 32) | Chi square |
| Sex                              |                              |                        |            |            |                        |            |
| Male                             | 123                          | 120                     | 0.002      | 23          | 21                      | 0.330      |
| Female                           | 64                            | 63                      | NS         | 16          | 11                      | NS         |
| Age (yrs)                        |                              |                          |            |            |                        |            |
| Up to 39                         | 11                            | 1                       | NS         | 2           | 0                       | NS         |
| 40–49                            | 27                            | 19                      | 15.996     | 3           | 5                       | 2.837      |
| 50–59                            | 61                            | 48                      | P < 0.01   | 14          | 10                      | NS         |
| 60–69                            | 63                            | 74                      | 3          | 5           | 5                       | NS         |
| Depth of invasion (histological) |                              |                          |            |            |                        |            |
| pT1                              | 42                            | 51                      | NS         | 0           | 0                       | NS         |
| pT2                              | 88                            | 78                      | 11         | 13          | 12                      | 19         |
| pT3                              | 40                            | 38                      | 27         | 19          | 18                      | 12         |
| pT4                              | 14                            | 11                      | 1          | 0           | 1                       | 0          |
| Unknown                          | 3                             | 5                       | 0          | 0           | 0                       | 0          |
| Nodal involvement (histological) |                              |                          |            |            |                        |            |
| pN0                              | 76                            | 79                      | NS         | 1           | 0                       | NS         |
| pN1                              | 67                            | 49                      | 18         | 12          | 18                      | 12         |
| pN2                              | 27                            | 29                      | 20         | 20          | 20                      | 20         |
| pN1                              | 11                            | 17                      | 0          | 0           | 0                       | 0          |
| Unknown                          | 6                             | 9                       |            |            |                        |            |
| Distant metastasis               |                              |                          |            |            |                        |            |
| M0                               | 157                           | 150                     | 39         | 32          | 32                      | 32         |
| Pathological stage               |                              |                          |            |            |                        |            |
| IA                               | 35                            | 40                      | NS         | –           | –                       | NS         |
| IB                               | 39                            | 38                      | –          | –           | –                       | –          |
| III                              | 38                            | 33                      | –          | –           | –                       | –          |
| IIIA                             | 30                            | 25                      | 30         | 25          | 30                      | 25         |
| IIIB                             | 9                             | 7                       | 9          | 7           | 9                       | 7          |
| IV                               | 31                            | 37                      | –          | –           | –                       | –          |
| Operation method                 |                              |                          |            |            |                        |            |
| Total                            | 42                            | 40                      | NS         | 12          | 11                      | NS         |
| Extended gastrectomy             | 141                           | 136                     | 27         | 21          | 27                      | 21         |
| Non-resectable                   | 4                             | 7                       | 0          | 0           | 0                       | 0          |

NS: not significant. According to the UICC classification (4th edition).

Incidence and site of recurrence

The sites of recurrence were identified during follow-up in 71 of the 370 patients who underwent curative resection. All types of recurrence were reduced in the OK-432 it group compared with the control group and both regional lymph nodal and haematogeneous recurrence were markedly reduced (Table 2).

Effect of stage, TILs grade, and extent of lymph node metastasis

OK-432 it significantly improved the survival rate of stage IIIA+IIIB patients (Figure 2). Among the patients who were negative for TILs, there was no difference in survival between the OK-432 it and the control groups. However, among TILs-positive patients, the OK-432 it group showed a 30% higher survival rate than the control group from the 2nd year after surgery (P = 0.0149). The survival rate of patients with pN2 was also higher in the OK-432 it group than in the control group (P = 0.0552) (Figure 3).

Relationship among survival rate, TILs grade, and extent of lymph node metastasis (Figure 4)

Among TILs-positive patients who had lymph node metastasis, OK-432 it was found to be most effective in improving the survival rate (P < 0.001).

Relationship between TILs grade and the frequency of metastatic lymph nodes

There was no difference between the OK-432 it group and the control group in the frequency of patients having metastatic lymph nodes. Although, the number of dissected lymph nodes per patient was similar in both groups, irrespective of the presence or absence of OK-432 it or TILs. However, a significant difference was found in the number of metastatic lymph nodes per patient. The ratio of metastatic nodes were markedly lower in the OK-432 it group with positive TILs than in the control group with positive TILs (Table 3). Only in the OK-432 it patients with positive TILs did the number of metastatic lymph nodes per patient decrease.
significantly in the regional through the distant node groups. The number of metastatic lymph nodes per patient in the regional node group was 2.11 in the OK-432 it patients with positive TILs, 4.47 in the OK-432 it patients with negative TILs, 4.62 in the control patients with TILs, and 4.72 in the control patients without TILs.

Relationship between OK-432 it and pattern of lymph node metastasis (Table 4)
The minor metastatic foci was smaller in the OK-432 it group than in the control group, particularly in the distant nodes from the main tumour than in the regional (perigastric and periarterial) nodes, suggesting that minor metastatic foci may be eliminated by OK-432 it.

Multivariate analysis of prognostic factors (Table 5)
8 variable factors, which were the extension of the primary tumour (pT), the extent of regional lymph node metastasis (pN), sex, age, venous invasion, lymphatic invasion and TILs, were selected by Cox’s regression analysis. In the control group, pN was the most important prognostic factor. On the other hand, in the OK-432 it group, TILs was a significant factor rather than pN.

Table 2 Incidence and site of recurrence of curatively resected cases

| Recurrence site       | OK-432 i.t (%) | Control (%) |
|-----------------------|---------------|------------|
| Brain                 | 4 (3.6)       | 3 (3.9)    |
| Liver                 | 11 (9.8)      | 18 (12.2)  |
| Lung                  | 6 (5.6)       | 10 (7.4)   |
| Vesseral type*        | 15 (30.6)     | 27 (57.4)  |
| Peritoneum            | 20 (13.2)     | 22 (15.0)  |
| Haematogenic          | 11 (9.8)      | 18 (12.2)  |
| Lymph node            | 4 (3.6)       | 9 (6.8)    |
| Liver                 | 6 (5.6)       | 10 (7.4)   |
| Lung                  | 1 (1.0)       | 4 (3.6)    |
| Bone                  | 4 (4.6)       | 3 (2.7)    |
| Brain                 | 0 (0.0)       | 1 (0.7)    |

Included multi-focal recurrence sites. *P = 0.034 by χ² test control vs. OK – 432 it.

DISCUSSION
The mortality rate from gastric cancer has been decreasing worldwide (Howson et al, 1986; Stat Bite, 1998), however, gastric cancer remains one of the major causes of death in Japan. Despite the progress of endoscopic diagnosis and surgical procedures in the last 3 decades, the survival rate after curative resection of advanced gastric cancer has remained low (The Japanese Gastric Cancer Study Group, 1981; Siewert et al, 1993). There has been and still is no established adjuvant chemotherapy for gastric cancer. Accordingly, various type of adjuvant chemotherapy and/or adjuvant cheimomunotherapy have been performed to improve the survival rate after operation for advanced gastric cancer. In those days of protocol planning, administration of intravenous MMC and oral tegafur (fluorouracil) had been the standard adjuvant chemotherapy for postoperative gastric cancer patients in Japan. Tegafur (Grem, 1996) is orally administered as a prodrug of 5-FU. Phase II trials in various solid tumours have indicated that tegafur has an activity equal to that of 5-FU. Adjuvant chemoinmunotherapy has been extensively performed by systemic administration of OK-432 for a variety of malignancies (Watanabe and Iwa, 1984; Cervical Cancer Immunotherapy Group, 1987; Arinaga et al, 1997). OK-432 is an immunomodulatory agent prepared from an avirulent human strain of Streptococcus pyogenes. It has been shown to induce various cytokines, especially Th1 cytokines (Saito et al, 1982; Yamamoto et al, 1986; Yang et al, 1992) and to upregulate both perforin (Kataoka et al, 1991) and Fas ligand expression (Toda et al, 1996; Sato et al, 1997) by OK-432 may play a major role in promoting anti-tumour necrotic and apoptotic effects of immune cells like NK cells, LAK cells, CTL (Kagii et al, 1994; Uchida and Micksha, 1983), which may be responsible for its therapeutic effect. The augmentation of perforin (Kataoka et al, 1991), induction of IL-2 and IFN-γ, and the upregulation of Fas ligand (Toda et al, 1996; Sato et al, 1997) by OK-432 may play a major role in promoting anti-tumour necrotic and apoptotic effects of immune cells like NK cells, LAK cells, CTL (Kagi et al, 1994; Lowin et al, 1994; Lin et al, 1995; Frost et al, 1997), and TILs. These such biological activity of OK-432 are thought to occur at the tumour site in the case of intratumoral injection of OK-432. In our previous studies (Gotoh et al, 1991). TILs induced by OK-432...
it showed augmented NK- and LAK activity, whereas TILs from patients without OK-432 it had no antitumor activity. Considerable destruction of tumour cells was also found in tumour specimens from patients with OK-432 it. Recently, Khalili et al (1998) reported that in ovarian cancer patients the spontaneous production of IL-10 and TNF-α were higher in TILs than in peripheral blood mononuclear cells, and the anti-CD3-induced IL-12 production was dramatically lower in TILs. These biased Th2-dominant cytokines might contribute to the anergic T cell function against tumour cells in TILs. IL-10 is also produced by various human solid tumours and could serve to blunt a natural or induced antitumor immune response in the tumour site (Maguri et al., 1997). Lopez et al (1998) also reported that TILs from breast cancer were defective both in proliferate response and IL-2 receptor mRNA expression but recovered when incubated in the presence of recombinant IL-2. These findings support that anergic TILs in gastric cancer patients may recover the antitumor activity in the presence of Th1-dominant cytokines including IL-2 and IFN-γ induced by OK-432 it. When dissected regional lymph nodes from OK-432 it it was stained with an anti-OK-432 antibody, macrophages containing OK-432 particles were found in the dissected nodes from peri-gastric to para-aortic (data not shown). We previously reported that lymph nodes and TILs from patients with OK-432 it also demonstrated NK- and LAK activity, but no antitumor activity was seen in the nodes from patients without OK-432 it (Gotoh K et al., 1991). In this study, we reconfirmed that the grade of TILs was significantly increased in the OK-432 it group. The antitumor activity of TILs and lymph nodes activated by OK-432 it may be responsible for improving the 10-year survival rate of patients with stage IIIA+IIIB with positive TILs, and with both positive TILs and positive node metastasis in the group of OK-432 it. Our follow-up study of recurrence showed that regional lymph node metastasis and haematogenous metastasis were significantly lower in the group with OK-432 it. The number of metastatic lymph nodes was markedly decreased in the OK-432 it patients with positive TILs. In our multivariate analysis of 8 prognostic factors, pN was the most important prognostic factor in the control group but it was not significant in the OK-432 it group. The number of dissected lymph nodes with micrometastasis was decreased in the OK-432 it group. Moreover, the number of metastatic lymph nodes per patient was significantly decreased in the OK-432 it patients with positive TILs. These findings suggest the possibility of elimination of micrometastatic foci in regional lymph nodes by OK-432 it. Because we previously reported that injected OK-432 distributed in the regional lymph nodes, lymphocytes of TILs or regional lymph nodes activated by OK-432 it may be responsible for improving the 10-year survival rate with preoperative endoscopically OK-432 intratumoral injection of OK-432 according to clinical stage.
Table 3  Relationship between grade of TILs and grade of lymph node metastasis

| TILs            | Metastatic incidence by case (%) | Metastatic incidence by node (%) |
|-----------------|---------------------------------|---------------------------------|
|                 | Metastatic cases/Whole cases    | Metastatic nodes/Whole nodes    |
| OK-432 i.t.     | Control                         | OK-432 i.t.                     |
| No–mild         | 57.5                             | 57.7                            |
| Moderate–marked | 58.8                             | 56.8                            |

OK-432 i.t. vs control of metastatic incidence by node: \( P < 0.001 \).

Table 4  Morphologic pattern of metastatic foci on lymph nodes

|               | OK-432 i.t. | Control |
|---------------|-------------|---------|
|               | Minor       | Major   | Minor       | Major   |
|               | Solitary    | Multiple | Solitary    | Multiple |
| Regional      |             |         |             |         |
| Perigastric   | 67          | 10      | 46          | 141     |
| Periarterial  | 20          | 2       | 12          | 13      |
| Distal        |             |         |             |         |
| Para-aortic   | 1           | 0       | 4           | 14      |
| Total         | 88          | 12      | 62          | 194     |

The ratio of minor type in OK-432 is significantly reduced than that in control \( P = 0.018 \).

Figure 3  Relationship between grade of extent of lymph node metastasis and survival rate for gastric cancer patients with and without preoperative intratumoral injection of OK-432.
Table 5  Multivariate analysis for survival risk

| Variables | Control Wald Chi-Square | P value | OK-432 it Wald Chi-Square | P value |
|-----------|-------------------------|---------|---------------------------|---------|
| pN        | 4.4967                  | 0.034   | 1.2152                    | 0.270   |
|           | (1.335: 1.022–1.743)    |         | (1.228:0.852–1.779)       |         |
| pT        | 4.0119                  | 0.045   | 5.0619                    | 0.025   |
|           | (1.431:1.008–2.032)     |         | (1.683:1.069–2.648)       |         |
| v         | 3.5587                  | 0.059   | 0.0126                    | 0.911   |
|           | (1.289:0.990–1.679)     |         | (0.980:0.694–1.386)       |         |
| ly        | 1.7196                  | 0.189   | 4.0778                    | 0.044   |
|           | (1.209: 0.910–1.606)    |         | (1.363: 1.009–1.841)      |         |
| INF       | 0.8629                  | 0.353   | 0.0628                    | 0.802   |
|           | (1.235: 0.791–1.928)    |         | (0.947:0.621–1.445)       |         |
| TIL       | 0.2830                  | 0.595   | 11.083                    | 0.001   |
|           | (1.181: 0.640–2.178)    |         | (0.329: 0.171–0.633)      |         |
| sex       | 0.2761                  | 0.599   | 0.003                     | 0.953   |
|           | (0.871: 0.520–1.459)    |         | (0.983: 0.557–1.735)      |         |
| age       | 0.0030                  | 0.956   | 5.196                     | 0.023   |
|           | (0.992: 0.738–1.333)    |         |                            |         |

v: the degree of venous invasion; ly: the degree of lymphatic invasion; INF: the pattern of infiltrating growth into the surrounding tissue. These classifications were according to Japanese Classification of Gastric Carcinoma (First English Edition).

Figure 4  Relationship among survival rate, TILs grade, and extent of lymph node metastasis
Advanced gastric cancer patients who are positive for TILs after OK-432 it should be considered as responders to OK-432, to whom adjuvant immunotherapy with intradermal injection of OK-432 should be continued in order to suppress recurrence over the long term, particularly in stage IIIA+IIIB.

The eradication of micro-metastasis is a most important strategy to improve the prognosis of cancer patients. Our study shows that preoperative intratumoral injection of OK-432 will be a more promising neoadjuvant immunotherapy for advanced gastric cancer patients.

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