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ORIGINAL ARTICLE

The efficacy of adjuvant immunochemotherapy with OK-432 after curative resection of gastric cancer: an individual patient data meta-analysis of randomized controlled trials

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Abstract
Background OK-432 has been used as a cancer treatment for 40 years, and the immunostimulatory effects of OK-432 therapy have been intensely investigated in Japan. Recently, it has received attention as a possible booster for cancer vaccine treatments. Our previous meta-analysis based on summary measures revealed a significant improvement in the survival of patients with curatively resected gastric cancer. However, it is impossible to exclude the possibility of bias due to several prognostic factors.
Methods We collected individual data for patients with stage III or stage IV gastric cancer after curative resection from 14 trials that were identified in a previous meta-analysis. Immunochemotherapy with OK-432 was compared with treatment with standard chemotherapy on an intention-to-treat basis. The primary end point was overall survival. Stratified survival analyses were performed with the trial as the stratification factor. Subgroup analyses were also performed according to the potential prognostic factors, which included pathological factors, splenectomy, and delayed-type hypersensitivity.
Results There were 796 and 726 patients in the OK-432 and control groups, respectively. The median overall survival was 42.6 months for the OK-432 group and 32.3 months for the control group. The overall hazard ratio was 0.88 (95 % confidence interval 0.77–1.00, $p = 0.050$). No factor showed a statistically significant interaction in the subgroup analyses.
Conclusions The results suggest that immunochemotherapy treatment with OK-432 could have a borderline significant effect for patients with stage III or stage IV gastric cancer after curative resection.

Keywords Adjuvant immunochemotherapy · Gastric cancer · OK-432 · Individual patient data · Meta-analysis

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Introduction

OK-432 is a lyophilized preparation produced by incubating a culture of the low-virulence Su strain of group A *Streptococcus pyogenes* of human origin that is treated with penicillin G potassium. It was approved in 1975 for clinical use in cancer patients by the Japanese Ministry of Health and Welfare [1], and was indicated for (1) prolongation of survival time in patients with gastric cancer or primary lung cancer in combination with chemotherapy [2, 3], (2) reduction of cancerous pleural effusions or ascites in patients with gastrointestinal or lung cancer [4, 5], (3) treatment of head and neck cancer and thyroid cancer resistant to other chemotherapies [6, 7], and (4) lymphangioma [8]. The antitumor activities of OK-432, in particular the immunostimulatory effects, have been

intensely investigated in Japan, but to a much lesser extent in the USA and Europe.

The results from several clinical studies that were conducted primarily in Japan were published between 1990 and 2000. Although favorable effects after treatment were reported in anecdotal studies, no individual randomized clinical trial demonstrated a significant improvement in survival. In 2002, we investigated the efficacy of adjuvant immunochemotherapy with OK-432 in 1522 patients with curatively resected gastric cancer, and demonstrated that standard chemotherapy combined with OK-432 treatment was superior to chemotherapy alone. The 3-year overall survival (OS) odds ratio was 0.81 (95 % confidence interval, CI, 0.65–0.99) [9]. Although the results demonstrated the benefits of combined treatment, the possibility of bias due to several prognostic factors could not be excluded since the study was performed on the basis of the tabulated data from a meta-analysis of randomized trials.

New aspects of OK-432 treatment have been investigated since the beginning of the twenty-first century, and multiple lines of evidence supporting the effects of OK-432 have been reported, including that (1) OK-432 induces dendritic cell maturation and induces cytotoxic T lymphocytes specific to tumors [10–12], (2) OK-432 is effective as an adjuvant to peptide vaccines such as NY-ESO-1 [13–17], and (3) OK-432 is effective as an adjuvant to cisplatin and hyperthermotherapy [18, 19]. In addition to the efficacy of adjuvant OK-432, it was also expected to overcome the suppression of regulatory T cells [20, 21], and to induce a helper T cell response [22]. With the increased attention on OK-432 therapy, a detailed reevaluation of the results of cancer therapy using OK-432 in previous clinical trials was determined to be important.

In this study, we collected data for individual patients who were enrolled in eligible randomized trials and reexamined the precise effects of immunotherapy using OK-432 in an adjuvant setting for locally advanced disease. The current standard of treatment for patients with stage I gastric cancer after curative resection does not necessarily involve adjuvant chemotherapy, and given the results of ACTS-GC [23], chemotherapy without immunotherapy is generally sufficient for stage II gastric cancer; however, more powerful therapy is required to treat stage III gastric cancer and curatively resected stage IV gastric cancer. In this regard, we focused on patients with stage III or stage IV curatively resected gastric cancer. This reanalysis was important in order to clarify the immunological effects of OK-432, which has become widely used as a new immunotherapy and vaccine therapy for various cancers.

Methods

We performed a literature search with the MEDLINE database, presentations at meeting and inquiry for the pharmaceutical industry and research groups in August 1999. Trials were eligible if (1) patients were randomized, (2) a curative resection was scheduled, (3) standard chemotherapy was performed after tumor resection, and (4) OK-432 was administered intramuscularly or intradermally. The PRISMA diagram in Fig. 1 shows this process, and additional details regarding the trials included have been published elsewhere [9].

The corresponding authors from each trial provided the individual patient data (IPD), including sex, age, pathological tumor–node–metastasis (TNM) stage, clinical stage,

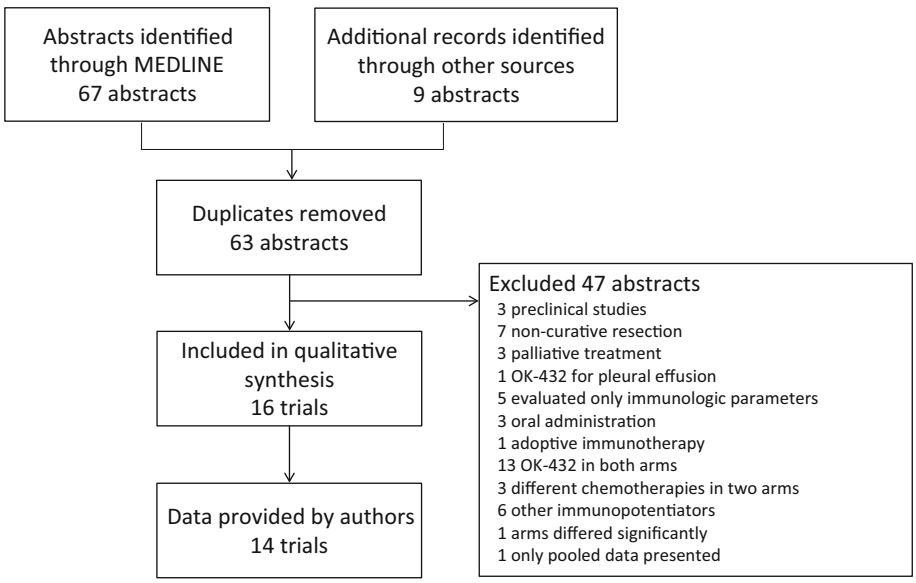

Fig. 1 Flowchart of the study design and trial selection

operation type, splenectomy status, delayed-type hypersensitivity reaction, date of operation, date of last follow-up, and survival status. The stage classification was performed according to the Japanese Classification of Gastric Carcinoma (13th edition). In this edition, stage IV cancer included a pathological T4 cancer without any metastasis as well as cancer with distant metastasis and para-aortic modal metastasis. In this IPD meta-analysis, all patients with stage III or stage IV gastric cancer were considered eligible, except for those with distant metastasis or para-aortic nodal metastasis. In addition, patients were excluded from the analysis if the data for survival duration or status were not available or if a noncurative resection had been performed. The primary end point was OS, which was defined as the time between the operation date and either the date of death from any cause or the last follow-up date. For surviving patients, the last follow-up date was treated as the censoring date.

Statistical methods

IPD meta-analysis is a type of analysis method for systematic reviews. We analyzed the IPD directly rather than extracting tabulated data from publications. The data were obtained directly from the investigators. Survival curves were estimated using the Kaplan–Meier method and were compared using a stratified log-rank test. A stratified proportional hazards model was used to estimate the hazard ratio (HR) and 95 % confidence interval (CI) for the effects of OK-432. The trial was the stratification factor. Cochran's Q and I^2 statistics for inconsistency [24] were used to assess statistical heterogeneity across the trials, and publication bias was assessed using a funnel plot. The effects of OK-432 in the different subgroups were investigated using stratified analysis with an interaction term. Forest plots were drawn to display the HRs by trial or by subgroup. A multivariate Cox model was fitted to the data to estimate the HR for OK-432, with adjustment for potential confounding factors found to be associated with OS ($p < 0.10$) in the univariate analyses. All p values were two-sided. All analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, USA) and Comprehensive Meta-Analysis (Biostat, Englewood, NJ, USA).

Results

We identified 67 abstracts from MEDLINE and nine abstracts from other sources. Abstracts from 16 studies were eligible (Fig. 1). The authors of 14 of the 16 eligible trials [9] were asked for and provided IPD ("Appendix"). We analyzed 1522 patients with stage III or stage IV gastric cancer. There were 796 patients in the OK-432 group and 726 patients in the control group. The numbers of patients in the OK-432 and control groups were well balanced in all trials. One trial was conducted in Korea, and the other 13 trials were conducted in Japan. Central randomization was

Table 1 Study design of randomized clinical trials using OK-432 in patients with stage III or stage IV gastric cancer

	Trial	Randomization	Chemotherapy	No. of patients	
				OK-432	Control
1A	Aichi Cancer Center Hospital	Envelope	MMC (iv) + 5-FU	14	19
3A	Kyoto Research Group for Digestive Organ Surgery	Envelope	MMC (iv) + 5-FU or FT-207 or HCFU	75	56
4A	JFMC (project #4)	Central	MMC (iv) + UFT	23	23
5A	JFMC (project #5)	Central	MMC (iv) + FT-207	108	88
6A	JFMC (project #17)	Central	MMC (iv) + UFT	188	171
7A	CSGIGC	Envelope	MMC (iv) + 5-FU	42	45
8A	Osaka OK-432 Study Group	Central	MMC (iv) + UFT	29	29
9A	Kyushu University	Envelope	MMC (iv) + UFT	12	12
11A	Kyung Hee University	Envelope	MMC (iv) + 5-FU (iv) +ADM (iv)	29	33
12A	Hokkaido University Group (study #2)	Central	MMC (iv) + HCFU	58	55
13A	Hokkaido University Group (study #3)	Central	5′-DFUR	49	37
14A	Ojiya General Hospital	Envelope	MMC (iv) + 5-FU or UFT	5	3
15A	Teikyo University	Envelope	MMC (iv) + UFT	7	3
16A	SPOG	Central	MMC (iv) + 5-FU	157	152
Total				796	726

ADM adriamycin, *CSGIGC* Clinical Study Group of Immunotherapy for Gastric Cancer, *5′-DFUR* doxifluridine, *FT-207* tegaful, *5-FU* 5-fluorouracil, *HCFU* carmoful, *iv* intravenously, *JFMC* Japanese Foundation for Multidisciplinary Treatment of Cancer, *MMC* mitomycin C, *SPOG* Study of Postoperative Adjuvant Immunochemotherapy using OK-432 for Gastric Cancer, *UFT* tegaful and uracil

Table 2 Patient characteristics

	OK-432	Control	*p*
Gender			
Male	539 (67.7 %)	475 (65.4 %)	0.345
Female	257 (32.3 %)	251 (34.6 %)	
Median age (years)[a]	60 (52–67)	61 (51–67)	0.535
pT			
T2	229 (28.8 %)	203 (28 %)	0.83
T3	484 (60.8 %)	441 (60.7 %)	
T4	83 (10.4 %)	82 (11.3 %)	
pN			
N0	15 (1.9 %)	17 (2.3 %)	0.322
N1	300 (37.7 %)	248 (34.2 %)	
N2	481 (60.4 %)	461 (63.5 %)	
M			
M0	796	726	
Stage			
III	766 (96.2 %)	685 (94.3 %)	0.083
IV	30 (3.8 %)	41 (5.7 %)	
Splenectomy			
No	536 (67.3 %)	458 (63.1 %)	0.082
Yes	260 (32.7 %)	268 (36.9 %)	
Delayed-type hypersensitivity			
Negative	170 (42.6 %)	148 (39.9 %)	0.445
Positive	229 (57.4 %)	223 (60.1 %)	
Unknown	397	355	

[a] The interquartile range is given in *parentheses*.

performed in seven trials. Some studies included a very small number of patients (Table 1).

The patient characteristics are listed in Table 2. There were 539 male patients (67.7 %) in the OK-432 group and 475 male patients (65.4 %) in the control group. The median age was 60 years, and was balanced between the groups. The other patient characteristics were also balanced between both groups (Table 2). The median follow-up duration was 35.0 months (interquartile range 16.0–61.0 months). The median duration of OS was slightly different between the two groups (42.6 months in the OK-432 group and 32.3 months in the control group). During the follow-up period, 456 patients in the OK-432 group and 449 patients in the control group died (Fig. 2).

The number of events in each group and the HRs are listed in Fig. 3. Only one study (no. 9A) demonstrated a significant benefit of OK-432 therapy. One study (no. 14A) was extremely small and had no event in the OK-432 group; therefore, the standard error of the HR could not be calculated, and this study did not contribute to the estimation of the overall HR. There was a significant difference between the two groups, with an overall HR of 0.88

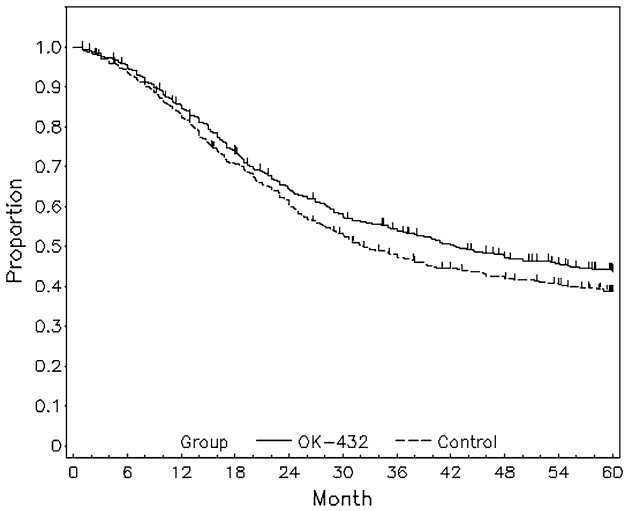

Fig. 2 Overall survival estimates after surgery truncated at 60 months. The survival rates were estimated using the Kaplan–Meier method

(95 % CI 0.77–1.00, $p = 0.050$). This corresponds to a 12 % reduction in the risk with OK-432 treatment. No significant heterogeneity of the treatment effects was detected across the trials ($p = 0.370$, $I^2 = 7.49$ %; Fig. 3).

A funnel plot is shown in Fig. 4. Horizontal and vertical axes are point estimates of the log HR and its standard errors of individual studies. Extremely small studies tended to have smaller HRs.

The subgroup analyses according to the prespecified prognostic factors are shown in Fig. 5. The observed OK-432 treatment effects were slightly greater in male patients than in female patients ($p = 0.188$). However, all factors showed no differences in the HRs between subgroups in interaction tests. The HR for OK-432 treatment based on centrally randomized trials (4A, 5A, 6A, 8A, 12A, 13A, and 16A) was not different from the overall estimate; this could hence be viewed as a sensitivity analysis, and indicated the robustness of the primary result. The covariate adjusted HR (0.91, 95 % CI 0.80–1.04), estimated by the multivariate Cox model with adjustment for pT, pN, stage, and splenectomy, was similar to the unadjusted value.

Discussion

Immunotherapy was once expected to become the ultimate treatment for various cancers, replacing the three major anticancer modalities of surgery, radiotherapy, and cytotoxic chemotherapy. However, most of the clinical trials evaluating the effects of immunopotentiators have failed to demonstrate a substantial benefit of treatment during the early phases of development. Beginning in the twenty-first century, molecularly targeted agents and monoclonal

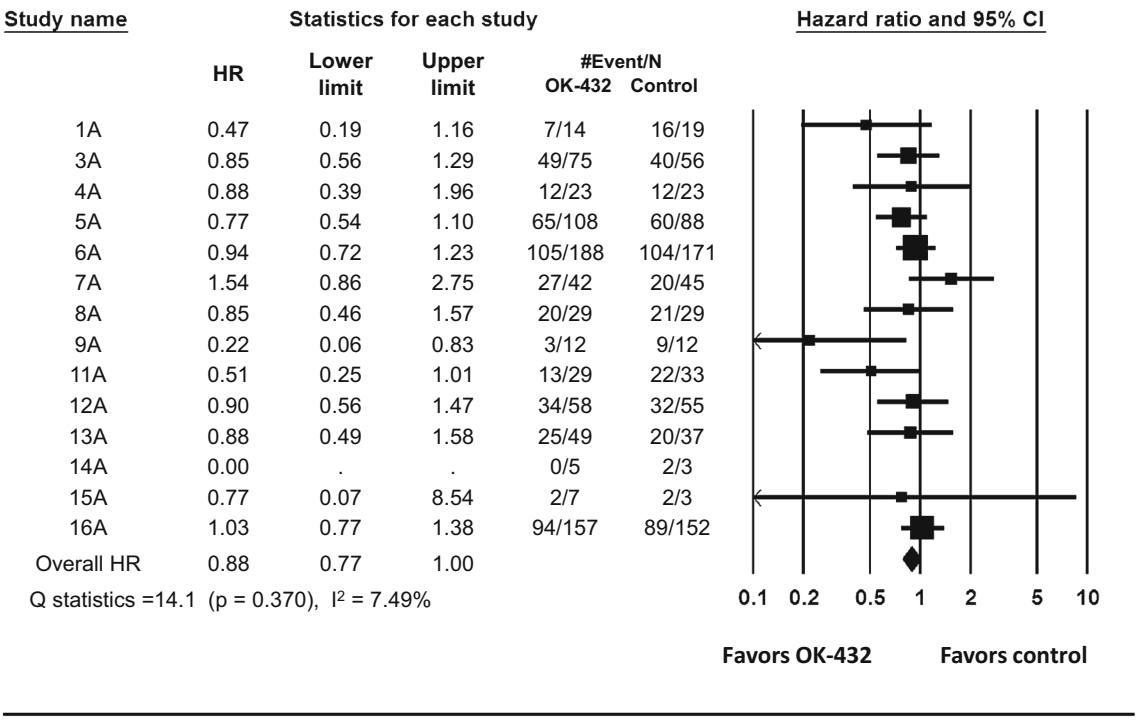

Fig. 3 Number of events and hazard ratios for the trials and the overall population. The *Q* statistic and the I^2 statistic were used to evaluate heterogeneity between trials. *Boxes* and *horizontal lines* in the forest plot represent point estimates, which vary in size according to the weight in the analysis, and 95 % confidence intervals, respectively. *CI* confidence interval, *HR* hazard ratio

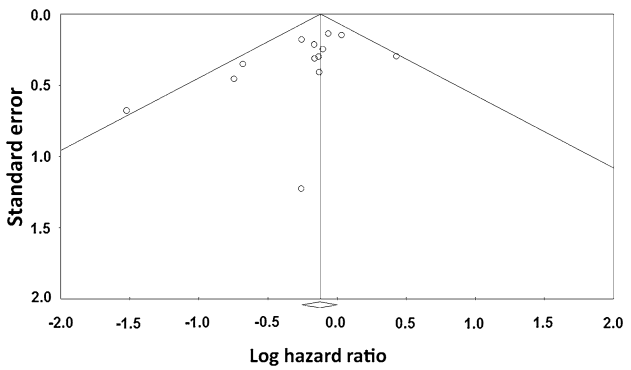

Fig. 4 Funnel plot depicting potential publication bias. The *horizontal axis* and the *vertical axis* reflect point estimates of the log hazard ratio and its standard error of individual studies, respectively

antibodies against specific cell or vascular endothelial growth factors, such as nivolumab and RG7446, were demonstrated to have clinical benefits in several cancer patients [25–30]. More recently, nonspecific immune activation systems have been shown to exert antitumor effects through direct intervention in the immune tolerance system. Given these new aspects of cancer immunotherapy, the importance of nonspecific immune activation by OK-432 in relation to the suggested new synergistic mechanism involving immunopotentiators and tumor vaccines has been highlighted. In this regard, a precise review of the previous clinical trials using OK-432 was considered essential for further investigation.

In this study, we performed the evidence synthesis of adjuvant immunochemotherapy with OK-432 treatment for stage III or stage IV gastric cancer without metastasis using IPD instead of tabulated data from previous analyses. The results revealed a 12 % risk reduction and a prolongation of survival (median 10 months), and suggested that the prognosis could be improved with this immunochemotherapy. Subgroup analysis of only the central randomized trials provided a similar result (HR 0.92, 95 % CI 0.79–1.06), demonstrating the robustness of the primary results regardless of the quality of the clinical trials.

In this research, we could have focused on the appropriate population, comprising stage III gastric cancer patients and curatively resected stage IV gastric cancer patients who required more powerful adjuvant treatment than chemotherapy alone. Generally, the reliability of the evidence from a subgroup analysis is not adequate in a single randomized controlled trial because of the problems of multiplicity and underpower due to the limited sample size. In this study with the large amount of IPD from 14 trials, we could investigate the precise effects of immunochemotherapy with OK-432. Furthermore, we could have tried to eliminate confounding and bias using a

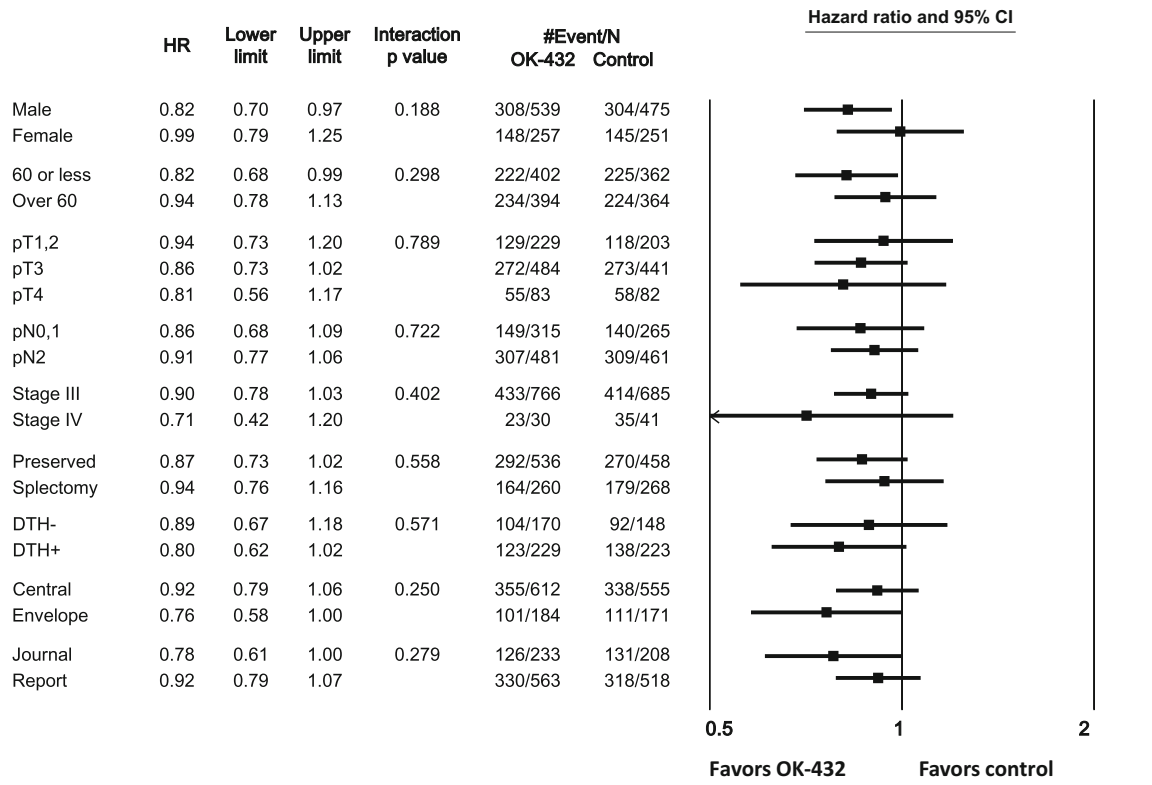

Fig. 5 Number of events and hazard ratios for the subgroups according to prognostic factors. The interaction *p* value represents heterogeneity between the subgroups. *Boxes* and *horizontal lines* in the forest plot represent point estimates, which vary in size according to the weight in the analysis, and 95 % confidence intervals, respectively. *CI* confidence interval, *DTH* delayed-type hypersensitivity, *HR* hazard ratio

multivariate analysis that adjusted for prognostic factors, and confirmed the robustness of the result.

In the subgroup analysis, the spleen-preserved group showed a higher HR than the splenectomy group, although the difference was not statistically significant. Some molecular findings support the hypothesis that the presence of the spleen enhances the antitumor immune response [31, 32]. For instance, Okinaga et al. [33] conducted a randomized trial to investigate the efficacy of spleen preservation and immunochemotherapy with OK-432 for gastric cancer patients. They reported that spleen preservation might be beneficial for the immunological function of patients with less-advanced gastric cancer. However, the differences in survival rates were not statistically significant, and the clinical benefit remains unclear.

Other immunochemotherapies have also been investigated for the treatment of gastric cancer. For example, Popiela et al. [34] evaluated the effects of adjuvant immunochemotherapy with the use of bacillus Calmette-Guérin, and 5-fluorouracil, adriamycin, and mitomycin C, and reported a 24 % risk reduction in patients with stage III or stage IV gastric cancer after curative resection. Oba et al. [35] performed a meta-analysis of immunochemotherapy with polysaccharide K for gastric cancer after curative resection, and reported a 12 % risk reduction. Fujimoto et al. [36] reported the effects of postoperative adjuvant immunochemotherapy with the polysaccharide sizofiran on the same population, and concluded that curative resection of gastric cancer resulted in a better prognosis when sizofiran was prescribed along with the antitumor drug.

In terms of the treatment of other cancers with OK-432, Sakamoto et al. [37] reported a significant benefit of OK-432 treatment for patients with resectable non-small-cell lung cancer. Watanabe et al. [38] conducted a randomized controlled tria involving patients with stage II–IV colorectal cancer. The results showed no significant differences in 5-year survival, but the combinations of OK-432 and an oral pyrimidine (1-hexylcarbamoyl-5-fluorouracil) or tegaful and uracil were well tolerated [38]. Most of the studies involving immunochemotherapy reported marginal results. This means that a larger clinical trial and simultaneous measurement of immunological parameters may be necessary to elucidate the underlying mechanism.

A limitation of this research was that the trials with fewer deaths tended to have smaller HRs. However, this is not likely due to publication bias because we performed a comprehensive literature search and obtained data from trials those were not published, and our research focused only on patients with stage III or stage IV gastric cancer among all the patients from the eligible trials. Further, data from two trials were not obtained. One of these trials reported the efficacy of OK-432 ($N = 236$ stage III gastric cancer patients, estimated HR 0.65), whereas the other did not ($N = 211$ stage II and stage III gastric cancer patients, estimated HR 1.12). If these data were included in the analysis, the overall HR would have become smaller. Finally, because the trials included in this research were completed over a decade ago, the surgical methods and treatments could have changed since then. However, we confirmed that no new clinical trial has been implemented, and believe that a detailed analysis of the old clinical trial data is of substantial importance, given that immunotherapy for cancer has been highlighted recently and OK-432 is one of the key substances as an adjuvant for vaccine therapy. In addition, the collection and registration of all the IPD will facilitate approaches for analyzing tissue specimens from patients enrolled in previous clinical trials that might be exploited for future research in the new era of immunotherapy.

In conclusion, this IPD meta-analysis suggests that immunochemotherapy with OK-432 could have a borderline significant effect for patients with stage III or stage IV gastric cancer after curative resection. Further investigations related to the new findings of immune tolerance systems and nonspecific immunopotentiators are warranted.

Acknowledgment This work was supported in part by the Epidemiological and Clinical Research Information Network, a nonprofit organization.

Appendix

The IPD were obtained from the following trials (the study number refers to Sakamoto et al. [9]). Data were provided by: Yoshitaka Yamamura (1A), Yoshinori Nio (3A), the Japanese Foundation for Multidisciplinary Treatment of Cancer and Hiroaki Nakazato (4A, 5A and 6A), Kenji Ogawa (7A), Tetsuo Taguchi (8A), Yoshiyuki Maehara (9A), Kim Si-Young (11A), Yuji Sato (12A and 13A), Tadahiro Yokomori (14A), Kota Okinaga (15A), and Chugai Pharmaceutical and Kunzo Orita (16A):

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3A Kyoto Research Group for Digestive Organ Surgery. A comprehensive multi-institutional study on postoperative adjuvant immunotherapy with oral streptococcal preparation OK-432 for patients after gastric cancer surgery. Ann Surg. 1992;216:44–54.

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