tomy depending on the tumor location and number of lymphatic basins: local resection, transactional gastrectomy, cardiac resection, and limited distal gastrectomy. To complete function-preserving curative gastrectomies, it is essential preoperatively to determine tumor status including tumor depth, size, and location using multimodal endoscopic examinations such as dye endoscopy and endoscopic ultrasonography. Furthermore, endoscopic sentinel node mapping by dye and radioisotope, and intraoperative frozen section biopsy of lymph nodes are required.

key words: function-preserving curative gastrectomy, sentinel node, lymphatic basin

Legends to Figures and a Table

Figure 1 What is an optimal choice between EMR/ESD and standard gastrectomy?
There is a great difference in the quality of life between the patients receiving EMR/ESD and standard gastrectomy. Japanese guidelines for gastric cancer treatment recommend D1+α/β operation for a substitute, but there is little difference in resected gastric extent between D1+α/β and D2 operation. We succeeded in reducing resected gastric extent by sentinel node mapping and selective lymphatic dissection. EMR/ESD+selective lymphatic dissection may be carried out in the future.

Figure 2 Gastric segment from the viewpoint of lymphatic flow.
a. Rouviere's classification. (Reference; Rouviere H: Anatomie des lymphatiques de l'homme. 294–334, Masson, Paris, 1932)
b. Coller's classification. (Reference No. 5)

Figure 3 PTD classification and lymphatic basins.
Border between zone-P and zone-T, level of bifurcation of upper and lower branches of left gastric artery on the lesser curvature, and level between left and right gastroepiploic arteries on the greater curvature; border between zone-T and zone-D, levels 8 cm apart from the pylorus on both the lesser and the greater curvatures.

Figure 4 Sentinel node mapping.
Tracers are endoscopically injected using an endoscopic injector into submucosa at four points around tumor where cancer is not proven preoperatively. Dye and radioisotope are injected by 0.1–0.2 m*l* and 0.5 m*l*, respectively, at each point.

Figure 5 NAVIGATOR GPS (United States Surgical, USA, supplied by Tyco Health Care Japan).

Figure 6 Blue node and lymphatic basin.
Blue node and blue lymphatics are defined as lymph node and lymphatics stained blue by patent blue, respectively. Lymphatic basin is defined as area including blue node and blue lymphatics, exisiting along a main gastric feeding artery.

Table Function-preserving curative gastrectomy combined with lymphatic basin dissection.

Ann Surg Oncol (2009) 16:1721
DOI 10.1245/s10434-009-0438-0

Annals of
SURGICAL ONCOLOGY
OFFICIAL JOURNAL OF THE SOCIETY OF SURGICAL ONCOLOGY

LETTER TO THE EDITOR

Reply: High Sensitivity of Indocyanine Green Fluorescence Imaging in Detection of Sentinel Node

TO THE EDITOR,

The argument by Takahashi et al. has been discussed in our article.[1] The following is our brief response.

Kitai et al. noted that the sensitivity of fluorescence spectroscopy is greater than that of absorption spectroscopy.[2] They also reported that it was possible to detect fluorescence with an indocyanine green (ICG) solution embedded 10 mm deep in a material that has optical properties like that of human tissue. In comparison, infrared rays can penetrate fatty tissues only to a depth of 3 mm. The difference could be critical in sentinel node detection. In this context, Ishikawa et al. reported an obese patient with a false-negative sentinel node when the infrared ray electronic endoscopy system was used.[3]

We reported that the ICG fluorescence imaging system was sensitive in both intraoperative ICG injection and ICG injection 1 day before surgery. We have argued that, similar to the radio-guided method, one advantage of preoperative tracer injection is that it eliminates the time-consuming intraoperative endoscopy, but has the disadvantage of loss of real-time tracing.[1]

An astral lamp, but not an ordinary light lamp, influences the ICG fluorescence imaging system. Therefore, surgery can be continued under an ordinary light lamp, which should not interfere with the ICG fluorescence imaging system. Furthermore, the light emitted by the laparoscope into the peritoneal cavity does not influence the ICG fluorescence imaging system. Laparoscopic exploration or surgery can be conducted under such an environment.

The laparoscopic system described in our report is not available commercially.[1] We have completed a preliminary study with a newly developed prototype system for laparoscopic surgery and are preparing a new report on its use in laparoscopic surgery.

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Published Online: 21 March 2009

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【ワンポイント・アドバイス】

胃癌におけるセンチネルリンパ節

宮代 勲 [大阪府立成人病センター消化器外科]

■腫瘍からのリンパ流を直接受けるリンパ節

腫瘍からのリンパ流を直接受けるリンパ節であるセンチネルリンパ節 sentinel node（SN）を同定することが可能であり，かつそこにリンパ節転移が認められなければ，SN以外のリンパ節には転移がないとして治療できるのではないか？ このコンセプトは，症例ごとにリンパ節転移有無を知るためのより確実・合理的な適応決定法として，悪性黒色腫や乳癌などの領域で臨床応用されている．

■リンパ節転移陰性を精度高く診断する指標

理論的には，リンパ節転移の可能性が非常に低い，もしくはなければ，後遺症を生じ得る胃切除やリンパ節郭清を予防的に行う意味はないと考えられるが，術前診断で得られた所見を過去のデータと照合することによりリンパ節転移がないであろう症例を割り出す現在の適応決定法では，根治性を保つために適応を厳しくせざるを得ない．リンパ節転移陰性を精度高く診断する指標の確立こそがブレークスルーとなる．根治性を損なうことなくリンパ節郭清を省略できれば，機能温存術式，腹腔鏡手術，内視鏡治療などへ展開できる．

■同定には適切なトレーサー注入が不可欠

SNの同定には適切なトレーサー注入が不可欠である．原発巣に近接して周囲を取り囲むように注入することが重要と考えられ，高度の線維化を伴う病変ではより注意を要する．トレーサーとしてradioisotope（RI）を用いる方法[1]は，放射線被曝，機器・試薬の取り扱いの煩雑さなどを伴い，取り扱いの容易な色素を用いる方法[2]は，経時的変化に弱い．色素のうち，isosulfan blue（Lymphazurin™）は日本で未承認であり，ショックを引き起こす可能性が低いとはいえない．異なる用途ではあるものの広く臨床で使用されているindocyanine green（ICG）は，リンパ系着色剤としての有用性が知られ，蛍光や赤外イメージングによる視認性向上の工夫も報告されている[3]．

■精度の高い術中迅速診断が求められる

SNの同定・摘出法には，pick up法やbasin法がある．また，basinではなくstationでの提唱もある．数個のリンパ節生検のみで転移診断を行うpick up法と異なり，basin法は支配動脈に伴走して5流域に分けられる胃のリンパ流のうち1～2流域を郭清するものである．もし，現行のリンパ節郭清を限られたリンパ流域のみの郭清に置換できるのであれば，nodeとしてのSNを同定・診断する必要はなく，lymphatic flowをみればよいことになるが，リンパ節郭清省略を目的とするSNコンセプトとの整合性が問われる．

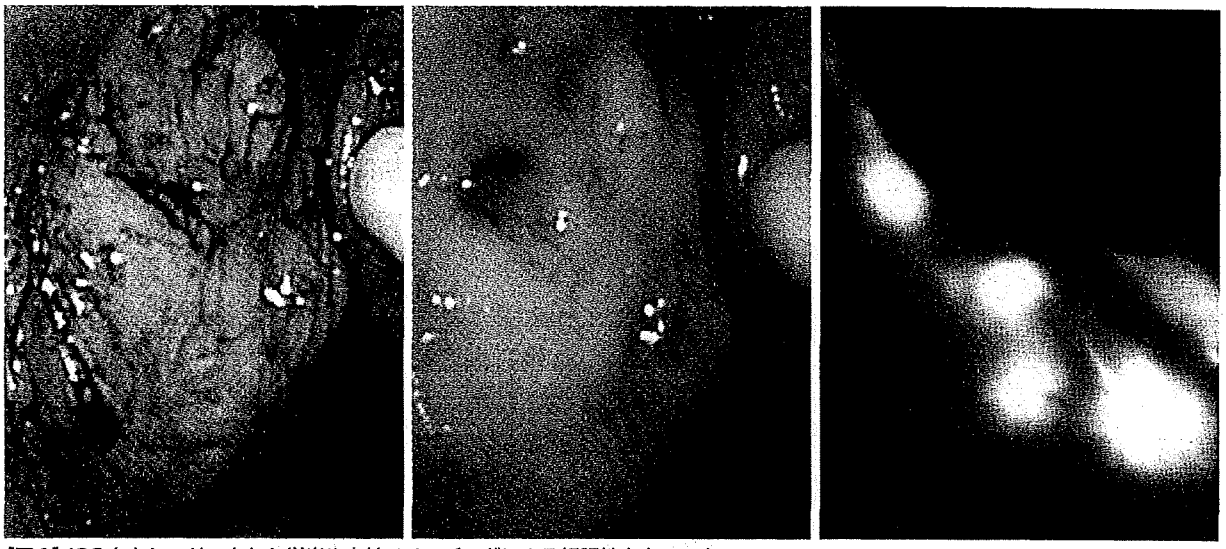
SNコンセプトをリンパ節郭清省略に臨床応用するには，胃切除前にリンパ節転移陰性を診断する必要があり，精度の高い術中迅速診断が求められる．病理部門の理解と協力が不可欠といえる．

■多施設共同研究による妥当性の検証

早期胃癌のリンパ節転移は少なく，妥当性検証には方法論を統一した多数例での検討を要する．日本臨床腫瘍研究グループJapan Clinical Oncology Group（JCOG）とSentinel Node Navigation Surgery（SNNS）研究会による二つの多施設共同研究が行われた．

JCOG胃がん外科グループによる「早期胃癌におけるセンチネルリンパ節生検の妥当性に関する研究（JCOG0302）」は，早期胃癌患者に対して，ICGを用いて同定されたgreen node（GN）をSNとみなし，GNの術中迅速病理診断でリンパ節転移陰性の場合にリンパ節郭清を行わないことの妥当性を評価することを目的とし，primary endpointは偽陰性割合（GN迅速病理診断転移陰性例/組織学的リンパ節転移陽性例）である．現時点で臨床応用した場合にどうなるかをみる設定であるため，SNコンセプトの妥当性のみならず，術中迅速病理診断およびラーニングカーブの問題などが包含されている点に留意すべきである．実際，偽陰性例の検討からこれらの問題が予想以上に大きいことが示唆されている．

SNNS研究会標準手技プロトコール作成委員会



[図1] ICGをトレーサーとした蛍光や赤外イメージングによる視認性向上の工夫

ICGの緑色だけ（左図）では同定しにくいリンパ節も，赤外イメージング（中央図）により認識しやすくなり，蛍光イメージング（右図）では4個と視認できる．（文献3）より引用一部改変）

による「胃癌におけるセンチネルリンパ節を指標としたリンパ節転移診断に関する臨床試験」は，RI法を基準とした胃癌におけるSNを指標としたリンパ節転移診断能を検証する試験でprimary endpointは転移検出感度（リンパ節郭清の結果で所属リンパ節に少なくとも1個以上のリンパ節に転移が認められた症例のうちSNに転移を有した症例の割合）である．例えば，同定・診断を切除前に限定していないなど，実臨床に応用するには課題が残るが，SNコンセプト自体をみる観点から，術中迅速病理診断やラーニングカーブの影響を受けにくいように設定されている．

胃癌におけるSNコンセプトは多施設共同研究によりその妥当性が検証されている段階であり，日常診療として安易に臨床応用すべき状況にはない．両試験には検証点の違いがあることに留意すべきであるが，いずれもSNコンセプトの胃癌治療への応用の可能性を検証する重要な試験であり，今後の展開が注目される．

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V 胃癌手術の Knack & Pitfalls

VI 腹腔鏡下手術の Knack & Pitfalls

[検印省略]

Knack & Pitfalls
胃外科の要点と盲点

定価(本体 18,000 円＋税)

2003年1月26日　第1版第1刷発行
2009年7月17日　第2版第1刷発行

監　修＝幕内雅敏
編　集＝荒井邦佳
発行者＝浅井宏祐
発行所＝株式会社 文光堂

〒113-0033 東京都文京区本郷7-2-7
電話　東京(03)3813-5478(営業)
東京(03)3813-5411(編集)

印刷所＝真興社

Printed in Japan

ISBN978-4-8306-2326-4

乱丁・落丁の際はお取り替えいたします

Molecular Detection of Lymph Node Metastases in Breast Cancer Patients: Results of a Multicenter Trial Using the One-Step Nucleic Acid Amplification Assay

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Abstract

Purpose: Accurate assessment of metastasis in sentinel lymph nodes (SLN) of breast cancer is important but involves a heavy workload for the pathologist. We conducted a multicenter clinical trial in Japan to evaluate a new automated assay system for cytokeratin 19 mRNA, the one-step nucleic acid amplification (OSNA) assay (Sysmex), to detect lymph node metastasis of breast cancer.

Experimental Design: Surgically obtained axillary lymph nodes were sectioned into four pieces, two of which were examined with the OSNA assay. The other two adjacent pieces were examined with H&E and immunohistochemical staining for cytokeratin 19. Serial sections at 0.2-mm intervals were used in trial 1 to determine the specificity of the OSNA assay, and three pairs of sections cut from the sliced surfaces of the pieces were used in trial 2 to compare the accuracy of the OSNA assay with that of a routine pathologic examination for SLNs in Japan.

Results: In trial 1, the sensitivity and specificity were 95.0% [95% confidence interval (95% CI), 75.1-99.9%] and 97.1% (95% CI, 91.8-99.4%), respectively, for 124 axillary lymph nodes obtained from 34 patients. In trial 2, the agreement between findings of the assay and of the pathologic examination was 92.9% (95% CI, 90.1-95.1%) for 450 axillary lymph nodes obtained from 164 patients.

Conclusion: The OSNA assay can detect lymph node metastasis as accurately as can conventional pathology and thus can be an effective addition to or alternative for rapid intraoperative examination of SLNs.

Sentinel lymph node (SLN) biopsy for breast cancer is expected to become a standard surgical procedure in the near future, and accurate assessment of metastasis of SLNs is essential for making decisions about the avoidance of unnecessary axillary dissection and the provision of appropriate adjuvant treatment for patients. However, methods for the pathologic examination of SLNs to detect metastasis remain controversial (1–4). Although more detailed examination of SLNs can provide more accurate information about metastasis (5), to obtain more accurate results, a comparatively greater number of pathologic specimens need to be examined (6). This involves much time for preparation of the specimens and a heavy workload for pathologists to examine them, especially intraoperatively.

To overcome these problems, molecular detection of metastasis has been developed as one of the most promising methods for SLN examination. With this procedure, the whole lymph node can be examined during a short time

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Received 7/20/08; revised 12/11/08; accepted 1/2/09; published OnlineFirst 4/7/09.
Grant support: Sysmex Corporation.
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Note: Y. Tamaki and F. Akiyama contributed equally to this work.
Sysmex Corporation contributed to providing the RD-100i system, funding of laboratory consumables for the OSNA assay, and collecting samples and data analysis but had no role in data interpretation and writing of the report.
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doi:10.1158/1078-0432.CCR-08-1881

Translational Relevance

Sentinel lymph node biopsy will most probably become a standard surgical procedure for early breast cancer patients; therefore, an accurate assessment of metastasis in sentinel node is required to avoid unnecessary axillary dissection. However, detailed examinations involve a heavy workload for the pathologists. Recently, several molecular detection procedures for lymph node metastasis have been developed as the most promising solution for this problem and now are commercially available. This paper reports the results of a Japanese multicenter clinical trial comparing a molecular-based method using a new automated assay system, the one-step nucleic acid amplification assay, with a routine pathologic examination for detection of lymph node metastasis of breast cancer. A high concordance rate was observed between the assay and the pathologic examination. The assay provided results in a short time and was easy to do. The one-step nucleic acid amplification assay may thus become an effective addition to or alternative for rapid intraoperative examination of sentinel lymph nodes.

without requiring much work for the pathologist. Very recently, several molecular-based metastasis detection procedures with proper calibration for clinical use have been developed and are expected to become alternatives to conventional pathologic examinations (7–10). The one-step nucleic acid amplification (OSNA) assay (Sysmex), an automated system for rapid and quantitative detection of cytokeratin 19 (CK19) mRNA with the reverse transcription loop-mediated isothermal amplification (RT-LAMP) method (11), has been shown to feature high specificity with a low false-positive rate (12). To assess the validity of this assay in clinical use, a multicenter clinical trial was conducted in Japan, concurrently with some single-institute studies in the Netherlands and Germany (13).

In this article, we report the results of the Japanese trial of the OSNA assay for detection of lymph node metastasis in breast cancer and discuss its validity for clinical use, especially for intraoperative SLN examination.

Materials and Methods

The two trials. The materials consisting of axillary lymph nodes were obtained from patients who underwent surgery for breast cancer between October 2005 and May 2006 at seven Japanese institutes and hospitals that had joined this study. Two of these institutes participated in trial 1, three in trial 2, and two in both. Patients were given the necessary information about the trial, and only the lymph nodes from patients who had given their consent were used. The trial consisted of two different protocols. In trial 1, designed to determine the specificity of the OSNA assay for detection of metastasis in comparison with that of detailed pathologic examination, clinically metastasis-negative (N0) lymph nodes with a maximum size between 4 and 8 mm were examined. Sampled lymph nodes were immediately divided into four pieces (a, b, c, and d) of 1 or 2 mm thickness with cutting devices developed by Tsujimoto et al. (12), and two of the four pieces (a and c) were examined with the OSNA assay in the laboratory of the participating institute concerned (Fig. 1). The two adjacent pieces (b and d) were sent to one of the three pathologists of the central committee, who examined them in the following manner. After the samples were fixed with formalin and embedded in paraffin, the two pieces were sliced sequentially at 0.2-mm intervals and a pair of sections with a thickness of 5 μm each was obtained from each level of slice. One of the paired sections was then stained with H&E, and the other was examined immunohistochemically for CK19 by using monoclonal antibody RCK108 (Dako). The pathologists examined the preps without access to information about the results of the OSNA assay. The pathologic diagnosis was negative when the specimen contained no tumor cells or only isolated tumor cells (ITC) and positive when micrometastasis or macrometastasis was found according to the criteria of the sixth edition of the tumor-node-metastasis classification of the International Union Against Cancer and the classification of the American Joint Committee on Cancer.

In trial 2, designed to determine the accuracy of the OSNA assay for detection of metastasis compared with that of routine pathologic examination, randomly sampled lymph nodes or SLNs with a maximum size between 4 and 8 mm were analyzed. The sampled lymph nodes were immediately divided into four pieces in the same manner as described above. Two pieces were examined with the OSNA assay, and the other two adjacent pieces were examined pathologically. Frozen sections of the SLNs were prepared and examined by a

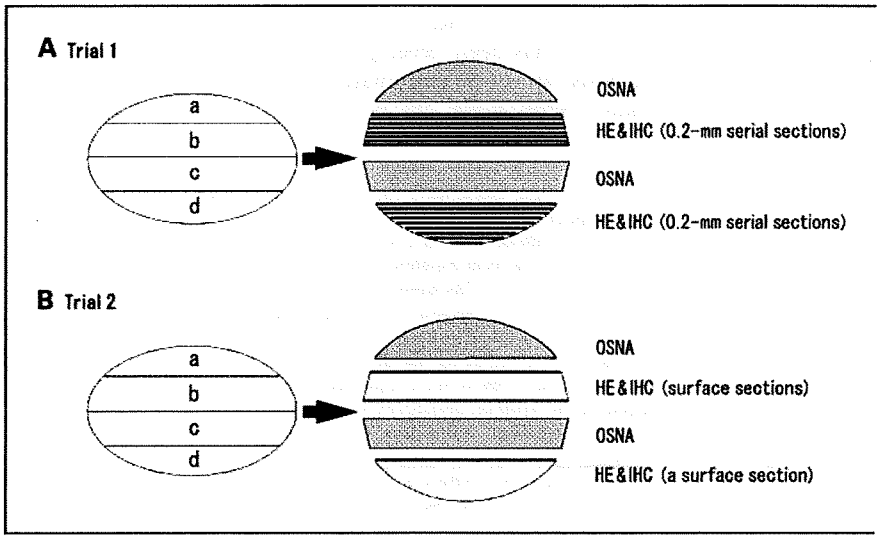

Fig. 1. Preparation of lymph nodes for the OSNA assay and pathologic examination. Lymph nodes were divided into four pieces (a, b, c, and d) 1 to 2 mm thick, and two pieces (a and c) were homogenized and subjected to the OSNA assay. In trial 1, the remaining two pieces (b and d) were serially sectioned at 0.2-mm intervals, and a pair of sections obtained at each level of the slice was stained with H&E and immunohistochemistry (*IHC*) for pathologic examination. In trial 2, a pair of sections obtained from the cut surface of the pieces was examined with H&E and immunohistochemical staining.

Table 1. Patient characteristics

	No. patients (%)	
	Trial 1	Trial 2
Enrolled	36	185
Excluded	2*	21†
Analyzed	34	164
Average age (y)	55.9	54.7
Clinical stage		
0	2 (6)	14 (9)
I	8 (24)	51 (31)
IIA	14 (41)	64 (40)
IIB	3 (9)	28 (17)
III	5 (15)	7 (4)
IV	0 (0)	0 (0)
Unknown	2 (6)	0 (0)
Histologic type		
DCIS	0 (0)	18 (11)
Invasive ductal	32 (94)	130 (79)
Invasive lobular	1 (3)	7 (4)
Special type	1 (3)	9 (5)

Abbreviation: DCIS, ductal carcinoma *in situ*.
*Consent was withdrawn by one patient and samples from another patient did not contain lymph node tissue.
† Consent was withdrawn by three patients and samples from four patients did not contain lymph node tissue. Six patients received neoadjuvant chemotherapy and the assay process was deemed invalid for eight.

pathologist at the institute concerned. The remains of the SLN specimens and intact non-SLN specimens were sent to the central committee where three pairs of sections were obtained at the cut surface of each piece (Fig. 1) and examined with H&E and immunohistochemistry for CK19 in the same manner as in trial 1.

The OSNA assay. The OSNA assay for lymph nodes has been described in detail in a previous report (12). Briefly, pieces obtained from axillary lymph nodes were homogenized with 4 mL of a lysis buffer solution and centrifuged at 10,000 × *g* at room temperature. Two microliters of the supernatant were analyzed with the RD-100i system (Sysmex), an automated molecular detection system using a RT-LAMP method. A standard positive control sample containing 5×10^3 copies/μL of CK19 mRNA and a negative control sample containing 0 copy/μL of CK19 mRNA were used for calibration in every assay. The results of the assay were expressed as the numbers of CK19 mRNA copies per microliter, and metastasis was assessed in accordance with the cutoff level determined by Tsujimoto et al. (12). That is, the lymph node was assessed negative when there were less than 2.5×10^2 copies/μL of CK19 mRNA and positive when there were 2.5×10^2 copies/μL or more.

Further examination for cases showing discrepancies between the OSNA assay and pathologic examination results. In trial 2, several nodes showed discrepant results for the OSNA assay and pathologic examination. After the trial period, such lymph nodes were subjected to further examination to determine the existence and localization of metastatic cells. For this purpose, the remaining pathologic specimen blocks were sectioned at 0.2-mm intervals and examined with H&E and immunohistochemistry for CK19 in the same manner as in trial 1. Furthermore, the lysate sample used for the OSNA assay was examined for CK19 protein expression by means of Western blotting analysis.

Statistical analysis. Sensitivity, specificity, and accuracy were determined by comparing the results of the OSNA assay and pathologic examination. The statistical program R 2.4.1[18] for binomial distribution analysis with 95% confidence interval (95% CI) was used for all statistical analyses.

[18] http://www.r-project.org/

Results

Trial 1. A total of 149 axillary lymph nodes surgically obtained from 36 patients with early breast cancer (Table 1) constituted the materials for this study. Five nodes from one patient were excluded from the analysis because consent was withdrawn by the patient, as were 19 nodes in which at least one of the four pieces did not contain lymphatic tissue and one involving a technical error. The remaining 124 nodes from 34 N0 patients were then analyzed. Of the 104 nodes pathologically identified as negative, 101 were assessed as negative by the OSNA assay for a specificity of 97.1% (95% CI, 91.8-99.4%; Table 2). Of the three nodes that were pathologically identified as negative but assessed as positive in the OSNA assay, one was found to contain ITCs and another to have >0.3 ng/μL of CK19 protein in the remaining sample solution used for the OSNA assay. Of the 20 pathologically positive nodes, 19 were assessed as positive by the OSNA assay for a sensitivity of 95% (95% CI, 75.1-99.9%). One node assessed as negative by the OSNA assay was found to contain a micrometastasis.

Trial 2. A total of 551 axillary lymph nodes surgically obtained from 185 patients with early breast cancer (Table 1) constituted the materials of this study. Eight of the nodes from three patients were excluded because their consent was withdrawn. Twenty-six nodes from six patients who received neoadjuvant chemotherapy were also excluded, as well as 36 in which at least one of the four pieces of a node did not contain lymphatic tissue and 31 that did not meet the specifications of this study, such as the use of frozen materials for the assay. Of the 450 lymph nodes eligible for the analysis, 70 were assessed as positive and 348 as negative by both the OSNA assay and pathologic examination for an accuracy of 92.9% (95% CI, 90.1-95.1%; Table 3). Seventy of the 80 pathologically positive nodes were detected by the OSNA assay (sensitivity, 87.5%; 95% CI, 78.5-93.8%). On the other hand, 348 of the 370 (94.1%) pathologically negative nodes were assessed as negative in the OSNA assay, whereas 5.9% of the pathologically negative nodes were positive. These lymph nodes showing discrepant results were subjected to further analysis.

Further examination of discrepant cases from trial 2. The 10 nodes that were pathologically positive but negative in the OSNA assay (false negative) and the 22 nodes that were

Table 2. Results of trial 1

	Pathology		
	Positive		Negative
	Macrometastasis	Micrometastasis	
OSNA			
Positive*	16	3	3
Negative†	0	1	101

NOTE: Specificity of the OSNA assay for pathology was 97.1% (101 of 104; 95% CI, 91.8-99.4%). Sensitivity of the OSNA assay for pathology was 95.0% (19 of 20; 95% CI, 75.1-99.9%).
*CK19 mRNA $\geq 2.5 \times 10^2$ copies/μL.
† CK19 mRNA $< 2.5 \times 10^2$ copies/μL.

Table 3. Results of trial 2

	Pathology		
	Positive		Negative
	Macrometastasis	Micrometastasis	
OSNA			
Positive*	64	6	22
Negative†	4	6	348

NOTE: Sensitivity of the OSNA assay for pathology was 87.5% (70 of 80; 95% CI, 78.2-93.8%). Specificity of the OSNA assay for pathology was 94.1% (348 of 370; 95% CI, 91.0-96.3%). Accuracy of the OSNA assay for pathology was 92.9% (418 of 450; 95% CI, 90.1-95.1%).
*CK19 mRNA $\geq 2.5 \times 10^2$ copies/μL.
†CK19 mRNA $< 2.5 \times 10^2$ copies/μL.

pathologically negative but positive in the OSNA assay (false positive) were subjected to further analysis. The results are summarized in Table 4. In eight of the 10 false-negative nodes, uneven localization of the tumor cells was found in the remnants of the nodes. However, two nodes with pathologically identified macrometastasis remained negative in the OSNA assay because tumor cells in these nodes showed faint expression of CK19, as was confirmed by immunohistochemical staining for CK19.

In 5 of the 22 false-positive nodes, some foci of tumor cells were found in the remnants of the nodes. Another eight of these nodes were not found to contain tumor cells in the pieces remaining after the pathologic examination, but lymphatic vascular invasions were detected in the main tumors of these nodes, whereas the lysate of two of them preserved for the OSNA assay contained a significant amount of CK19 protein. However, further analysis of the remaining nine nodes showed no pathologic or clinical signs of metastasis after additional sectioning.

The results based on the further analysis are shown in Table 5. The final accuracy of the OSNA assay based on the results of further examination was 93.1% (95% CI, 90.0-95.5%), the final sensitivity was 87.7% (95% CI, 78.5-93.9%), the final specificity was 94.3% (95% CI, 95.3-98.8%), the final positive predictive value was 77.2% (95% CI, 67.2-85.3%), and the final negative predictive value was 97.2% (95% CI, 95.5-98.9%). The OSNA assay detected 94.1% of the lymph node metastases larger than 2 mm (macrometastasis).

Table 4. Results of further analysis of lymph nodes with discrepant results in trial 2

No. LN	Result of trial 2		Result of further analysis	
	OSNA*	Pathology	CK19 protein (ng/μL)	Pathology
1	-	Ma	0.05	CK19 negative
2	-	Ma	0.14	CK19 negative
3	-	Mi	0.2	Mi in one of two pieces
4	-	Mi	0.13	Mi in one of two pieces
5	-	Mi	0.03	Mi in one of two pieces
6	-	Mi	0.14	Mi in one of two pieces
7	-	Ma	0.02	Ma in one and Mi in the other piece
8	-	Ma	0.26	Ma in one and Mi in the other piece
9	-	Mi	0.28	Mi in both pieces
10	-	Mi	0.04	Mi in both pieces
11	+	Neg (ITC)	0.32	Mi in one of two pieces
12	+	Neg (ITC)	0.3	ITC in one of two pieces
13	+	Neg (ITC)	0.04	ITC in one of two pieces
14	+	Neg (ITC)	0.08	ITC in one of two pieces
15	+	Neg (none)	0.04	ITC in one of two pieces
16	+	Neg (none)	0.38	None, ly+
17	+	Neg (none)	0.58	None, ly+
18	+	Neg (none)	0.08	None, ly+
19	+	Neg (none)	0.02	None, ly+
20	+	Neg (none)	0.12	None, ly+
21	+	Neg (none)	0.02	None, ly+
22	+	Neg (none)	0.05	None, ly+
23	+	Neg (none)	0.04	None, ly+
24	+	Neg (none)	0.1	None
25	+	Neg (none)	0.15	None
26	+	Neg (none)	0.02	None
27	+	Neg (none)	0.02	None
28	+	Neg (none)	0.1	None
29	+	Neg (none)	0.09	None
30	+	Neg (none)	0.15	None
31	+	Neg (none)	0.15	None
32	+	Neg (none)	0.03	None

Abbreviations: Ma, macrometastasis; Mi, micrometastasis; Neg, negative; none, no tumor cells; ly+, lymphatic vessel invasion observed in the main tumor.
*++: CK19 mRNA $\geq 5 \times 10^3$ copies/μL; +: $2.5 \times 10^2 \leq$ CK19 mRNA $< 5 \times 10^3$ copies/μL; -: CK19 mRNA $< 2.5 \times 10^2$ copies/μL.

Table 5. The final results of trial 2

	Pathology		
	Macrometastasis	Micrometastasis	Negative
OSNA			
Positive*	64	7	21
Negative†	4	6	348

NOTE: The final sensitivity of the OSNA assay for pathology was 87.7% (71 of 81; 95% CI, 78.5-93.9%). The final specificity of the OSNA assay for pathology was 94.3% (348 of 369; 95% CI, 95.3-98.8%). The final accuracy of the OSNA assay for pathology was 93.1% (419 of 450; 95% CI, 90.0-95.5%). The final sensitivity of the OSNA assay for pathologic macrometastasis (>2 mm) was 94.1% (64 of 68; 95% CI, 85.6-98.4%).
*CK19 mRNA $\geq 2.5 \times 10^2$ copies/μL.
†CK19 mRNA $< 2.5 \times 10^2$ copies/μL.

Discussion

Because several studies have shown the feasibility of molecular detection of micrometastasis in the lymph nodes using reverse transcription-PCR (14–17), a lot of markers, such as CK19, mammaglobin, carcinoembryonic acid, MUC1, prolactin-induced protein, have been examined for their sensitivity and specificity for detection of metastasis, and a variety of combinations of these markers have been proposed for clinical use (18–23). One of these, a combination of CK19 and mammaglobin, showed both high sensitivity and specificity for detection of lymph node metastasis of breast cancer (23), and the system using these two markers showed high reliability and is now being used clinically (7–10).

The OSNA assay is also a molecular-based metastasis detection system, which uses CK19 as a single marker. CK19, a representative epithelial marker widely expressed in human cancers, is considered to be a promising marker with high sensitivity for detection of lymph node metastasis from various cancers. Reverse transcription-PCR for CK19, on the other hand, is sometimes unreliable because of the presence of pseudogenes (24) and contamination of benign epithelial cells (25). To overcome this problem, the OSNA assay adopted the RT-LAMP method developed by Notomi et al. (11). The amplification is processed isothermally by means of six primers and can detect mRNA of CK19 quantitatively without interference by pseudogenes. The assay can differentiate contamination of a few benign epithelial cells and the presence of ITCs from clinically significant tumor metastasis by using a verified cutoff value (12). The assay can be done with on-the-spot preparation and easy operation because homogenization of a lymph node in the lysis buffer takes only 90 seconds and centrifugation of the sample 1 minute, whereas placement of the supernatant and reagents into the detector does not require extraction and purification of mRNA to synthesize cDNA, both of which are necessary for the reverse transcription-PCR method. The mRNA is automatically amplified in a RD-100i gene amplification detector (Sysmex) in 16 minutes, and stable results are provided without being affected by the size of the sample (maximum of 600 mg for one assay; ref. 12).

Trial 1 was designed to determine its specificity because high specificity is needed to avoid unnecessary axillary dissection when the assay is used for SLN examination. The specificity of the assay in our study was 97.1% (95% CI, 91.8-99.4%), with only three nodes judged positive in the OSNA assay but negative pathologically, but two of these nodes were found to contain ITCs in the specimen used for pathology. The specificity of the OSNA assay thus reaches 99.0% when these cases with ITCs are excluded.

In trial 2, the accuracy of the assay was examined in comparison with a routine method for pathologic examination for SLNs used in Japan, which is similar to a protocol with three sections from each 2- to 3-mm slice of the node recommended by the consensus meeting (26). The rate of concordance between the assay and the pathologic examination was 92.9% (418 of 450; 95% CI, 90.1-95.1%), and the results indicated that the lymph node metastasis detection capability of the OSNA assay was statistically equal to that of the pathologic examination for three sections cut from slices obtained at 2-mm intervals when discrepancies caused by differences in the samples used for the assay and the pathologic examination are taken into consideration. This is so because a sample for molecular examination needs to be homogenized, it cannot be used for pathologic examination, so that studies comparing the two modalities using different pieces of a sample must therefore of necessity include some cases producing discrepant results caused by uneven localization of tumor foci. Actually, 32 nodes (7.1%) with discrepant results were found. In eight of the 10 false-negative nodes, only a few of the serial sections contained metastasized foci of the tumor. These small metastases might have been detected by the OSNA assay if the whole lymph node had been used for the assay. In addition, some tumor cell clusters or ITCs were found in the remaining specimens of 5 of the 22 false-positive nodes after additional sectioning, which had not been detected by routine pathologic examination using 2-mm interval sections. In another eight false-positive nodes, no tumor cells were found in the pieces remaining after the pathologic examination, but lymphatic vascular invasions were observed in the main tumors of these nodes. In addition, the lysate of two of them preserved for the OSNA assay contained a significant amount of CK19 protein. These nodes may thus have harbored some foci of tumor cells in the piece used for the OSNA assay.

On the other hand, two nodes from different patients accounted for the false-negative cases with a very weak expression of CK19 mRNA. The primary tumors of the patients also showed negative staining for CK19 as confirmed by immunohistochemistry. The actual incidence of tumors with low CK19 expression remains unclear. Bartek et al. (27) reported an incidence for breast cancer of 0%, but Parikh et al. (28) of 20.5% for young patients. The OSNA assay is more sensitive than immunohistochemistry so that the incidence of false-negative results caused by low expression of CK19 can be expected to become exceptional in clinical use. In fact, another lymph node obtained from one of the two patients was positive in the OSNA assay, so that only 1 of the 185 patients (0.5%) was identified as negative by the assay. However, this ratio should be confirmed with more lymph nodes and patients.

The findings of the further analysis of discrepant cases in trial 2 prompted a reanalysis of the data, resulting in the final specificity of the OSNA assay compared with the pathologic examination becoming 94.3%, the final accuracy 93.1%, and the final negative predictive value 97.2%. These ratios indicate that the OSNA assay can accurately detect node-negative cases.

As for pathologically positive lymph nodes (diameter of metastasis, >0.2 mm), 87.7% could be detected by the OSNA assay, whereas 94.1% of macrometastases (>2 mm) were assessed as positive. Our results were similar to recently reported results reported by Visser et al. (13), who compared the OSNA assay with pathologic examination at five levels at 0.25-mm intervals of each piece used for pathology.

In conclusion, the OSNA assay showed high specificity, accuracy, and negative predictive value compared with conventional pathologic examination for the detection of lymph node metastasis of breast cancer. It provides satisfactory results in a short time and with easy procedure. The OSNA assay can thus be used as an alternative tool for examining metastasis in SLNs. However, for the time being, it is recommended to use the assay together with pathologic examination for minimal numbers of specimens until additional clinical trials with more lymph nodes and patients show that prognostic outcomes determined by the assay are equal or superior to those determined by conventional pathology.

Disclosure of Potential Conflicts of Interest

Y. Tamaki, F. Akiyama, T. Kaneko, K. Tsugawa, M. Tsujimoto, and N. Matsuura: honorarium, Sysmex Corp. S. Noguchi and N. Matsuura: Advisory Board, Sysmex Corp. M. Tsujimoto, T. Kaneko, and N. Matsuura: travel grant, Sysmex Corp.

Acknowledgments

We thank all the staff of the clinical and pathologic laboratories at the participating institutes for their technical support.

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Int J Clin Oncol (2009) 14:83–84
DOI 10.1007/s10147-009-0887-2

INTRODUCTION TO REVIEW ARTICLES

Junichi Sakamoto

Implementation and limitations of meta-analysis of randomized trials from the clinical biostatistician's point of view

Received: February 16, 2009

Despite the fact that a meta-analysis (also called an overview) is a conceptually simple process consisting of reanalyzing the data or the results of several independent experiments, its serious implementation in medicine began only at the end of the 1980s. In early breast cancer, significant survival benefits of adjuvant tamoxifen and cytotoxic therapy were established beyond a reasonable doubt, for the first time, by the Early Breast Cancer Trialists' Collaborative Group (EBCTCG). The first publication from that group included 61 randomized trials among 28 896 patients.[1] Of note, all analyses were based on carefully checked individual patient data. In colorectal cancer, in contrast, a meta-analysis based on summary data from large adjuvant trials of chemotherapy and radiotherapy could not reach a conclusion about the presence or absence of worthwhile survival benefits,[2] and the authors called for further large trials and meta-analyses to be conducted.[3] Encouraged by the success of the EBCTCG in early breast cancer, several meta-analysis groups were established in order to detect or confirm the existence of "moderate but humanly worthwhile" treatment benefits. In early breast cancer, the meta-analysis conducted by the EBCTCG has been continuously updated and its results published every 5 years. The latest publication included 194 clinical trials among 14 5000 women with breast cancer.[4] In colorectal cancer, the Meta-Analysis Group in Cancer (MAGIC) and the Colorectal Cancer Collaborative Group have collected individual patient data since the 1990s, with several important publications for both advanced and curatively resected colorectal cancers.[5,6]

Doubt still prevails among some clinicians about the credibility of the results of meta-analysis. One reason for such skepticism is based on the presumption that a large randomized trial is better than a meta-analysis of many small trials that differ in many respects and which, therefore, cannot be meaningfully combined, even if analyses are stratified by trial. Advocates of large trials claim that only such trials can give conclusive answers to a major clinical question.[7,8] However, these "definitive" trials would typically require several thousand patients, with the corresponding need for massive databases, the maintenance of high-quality data across many centers and often many countries, and therefore huge budgets that are not always available. Several trials looking at related questions may be easier to organize and more cost-effective, leaving a meta-analysis as the best way to combine all the available evidence and draw reliable conclusions about the treatment benefits and harms.

Several large trials for patients with colorectal tumors have recently been implemented in the United States, Europe, and Japan. Nonetheless, most randomized clinical trials performed in the world are still relatively small-sized, with the number of patients rarely exceeding 500, and as such these trials are underpowered and insufficient from a statistical point of view. Do we have to believe the results of such small-sized randomized trials? Probably not, because negative results might result from the inadequate sample size, while positive results would tend to be prominently reported and published sooner than negative results, a phenomenon known as publication bias. Last but not least, some of the trials may be affected by methodological flaws that may or may not be obvious from their published reports. It is unlikely that all trials are subject to similar flaws, which is why the replication of an experiment is such an essential principle in science generally, and in clinical research in particular.

Meta-analyses that are likely to yield reliable answers to important clinical questions should be based on two key principles. First, they should use individual patient data obtained from the principal investigators of all trials included in the meta-analysis.[9] Second, they should use all relevant trials and not just those that happen to be published or are otherwise available.[10–12] Investigators must also be aware of meta-analyses that intentionally eliminate inconvenient data and that may appear respectable, but may lead to seriously misleading results.[13,14]

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A major step towards making the meta-analytic process easier, more transparent, and more reliable, is the statutory clinical trial registration system that was introduced in the United States in 1997. In 2005, the International Council of Medical Journal Editors (ICMJE) adopted a policy requiring that studies be registered in order to be eligible for publication. In the ICMJE policy, a trial must have a prospectively assigned concurrent control or comparison group to trigger the requirement for registration.[15] Clinical trials, whether implemented by a pharmaceutical company, by a group of investigators, or by a single investigator, now have to be equally and prospectively registered in order to be published in quality medical journals. Hence, most if not all trials started after 2005 will be prospectively registered, which will greatly reduce the opportunity for publication bias by making unpublished trials visible (although their results may still take longer to be known).

In this special issue of the *International Journal of Clinical Oncology,* four distinguished expert clinical biostatisticians illustrate the various contributions and roles of meta-analysis; they discuss the implications of subset analyses to yield reliable answers to important clinical questions; and they describe an important initiative recently launched to facilitate future meta-analyses in gastric cancer (GASTRIC, for Global Advanced/Adjuvant Stomach Tumor Research through International Collaboration).[16,17] I hope that these review articles will provide clinicians with a more comprehensive understanding of the meta-analysis of randomized trials, especially of those trials for the treatment of solid tumors of the gastrointestinal tract.

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Gastric Cancer (2009) 12: 69–78
DOI 10.1007/s10120-009-0505-z

Review article

Paclitaxel chemotherapy for the treatment of gastric cancer

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Abstract
A comprehensive review of phase I and phase II clinical trials of paclitaxel and paclitaxel-containing chemotherapy regimens for advanced gastric cancer was performed. Response rates, median progression-free survivals, and median overall survivals were examined, together with the treatment regimens and the numbers of patients registered in each trial. Although paclitaxel monotherapy produced considerable improvement in tumor response and prognosis, combination doublet or triplet chemotherapy with fluoropyrimidines and/or platinum compounds showed better results than the paclitaxel monotherapy. With regard to the schedule of paclitaxel administration, weekly injection seemed to show less toxicity and better results than administration every 3 weeks. Adjuvant therapies, chemoradiation therapies, and paclitaxel treatment for gastric ascites were also investigated and are discussed.

Key words Paclitaxel · Gastric cancer · Chemotherapy

Introduction

Gastric cancer is one of the most common types of solid tumor, and it is estimated to be the fourth most common in terms of morbidity, and the second most frequent cause of cancer death in the world [1]. Gastric cancer is particularly common in Asia, eastern Europe, and in South America, where the preservation of food is mostly performed by submerging it into salt, and where the detection rate of *Helicobactor pylori* is considerably high.

In Japan, where a vast store of data is available because of the long-term effort of the Gastric Cancer Registry, gastric cancer is the second most common cause of cancer mortality. Although the incidence of gastric cancer has been declining in most developed countries, esophago-gastric junctional tumor and tumor in the cardia has, conversely, been increasing [2] and these tumors still remain one of the biggest problems worldwide.

The prognosis of patients with advanced (i.e., unresectable or metastatic) gastric cancer is very poor. The median survival time for such patients is 6 to 9 months [3]. For many years, various chemotherapeutic agents have been used in attempts to improve survival, progression free-survival, response rate, and quality of life in patients with advanced gastric cancer as well as to improve disease-free survival in patients in whom curative resection of the cancers has been performed. 5-Fluorouracil (5-FU) and cisplatin-based regimens have long been considered reference treatments. Commonly used regimens have included epirubicin, cisplatin, and continuous infusion of 5-FU; 5 days' infusion of 5-FU plus cisplatin every 4 weeks; a weekly infusion regimen of 5-FU/leucovorin (LV) over 24 h plus cisplatin every 2 weeks; and 5-FU bolus plus 22-h infusion of 5-FU on days 1 and 2, in combination with cisplatin every 2 weeks. The results with these regimens, together with other study results, suggested that combination regimens including fluorinated pyrimidines, cisplatin, doxorubicin, epirubicin, and methotrexate, had better response rates than single agents. Although gastric cancer is a relatively chemosensitive disease, with response rates of 30% to 40%, these treatments have shown a modest but unsatisfactory increase in overall survival [4]. In this regard, chemotherapy in the advanced gastric cancer setting is limited by a low complete response rate, response durations that are short-lived, and considerable toxicities.

Nevertheless, recently, the development of new chemotherapeutic and molecular targeting agents has opened the door to various clinical trials to find novel therapeutic strategies to improve the outcome of

Offprint requests to: J. Sakamoto
Received: February 4, 2009 / Accepted: March 21, 2009

patients with gastric cancer. Among such newly developed chemotherapeutic agents, paclitaxel has emerged as one of the most powerful compounds. Paclitaxel has activity against a broad range of tumor types, including breast, ovarian, lung, and head and neck cancers. Paclitaxel is also assumed to have activity in other malignancies that are refractory to conventional, first-line standard chemotherapies.

In this review, we focus on the activity of paclitaxel against advanced gastric cancers mainly through evidence-based medicine-oriented clinical trial results, and we evaluate the efficacy of combination chemotherapies, neoadjuvant and adjuvant chemotherapies, and multidisciplinary treatment with radiation therapy using paclitaxel.

Cytological and genetic reactions of paclitaxel in cancer cells

Paclitaxel, one of the taxanes, represents a new type of agent having both a specific chemical structure and mechanism of action.

Paclitaxel was discovered as part of the National Cancer Institute (NCI) national program in which thousands of plants, bacteria, and fungi were screened for the presence of anticancer activity. A crude extract from the bark of the Pacific yew, *Taxus brevifolia*, a slow-growing evergreen found in the Pacific northwest, proved to have cytotoxic activity against many cancer cells. Paclitaxel was obtained from the extract of the plant as an active constituent against cancer [5]. Although the development of paclitaxel was first disturbed by the scarce drug supply obtained from scarce natural products, semisynthetic replacement from other inactive precursor taxanes provided more abundant supplies.

Paclitaxel is an alkaloid ester consisting of a taxane ring system linked to a four-membered oxetan ring at positions C-4 and -5 (Fig. 1). Paclitaxel promotes the polymerization of tubulin, the principal function of tubulin being the formation of the mitotic spindle during cell division. Microtubules formed in the presence of paclitaxel are firmly stable and dysfunctional, thereby disrupting the normal microtubule dynamics required for cell division and interphase processes [6, 7]. Paclitaxel also induces the cellular process that leads to apoptosis or programmed cell death, even at doses that do not induce tubulin polymerization. Although the precise mechanism of this effect of paclitaxel has not yet been determined, cells exit from mitosis but do not continue to divide, and then substantial DNA fragmentation, indicative of apoptosis, leads to cell death in 2 to 3 days [8, 9]. The induction of tumor necrosis factor-α (*TNF-α*) gene expression is also caused by the action of paclitaxel, unrelated to its effect on microtubule assembly, raising the issue that this cytokine is related to the antitumor activity of paclitaxel [10]. This effect was not observed with other taxanes, such as docetaxel, although the clinical consequences of these differences have not been determined.

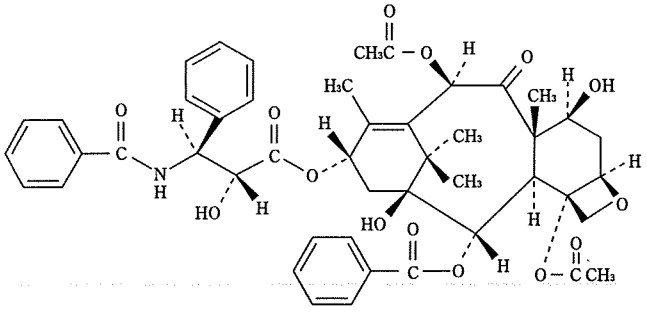

Fig. 1. The chemical structure of paclitaxel

Two mechanisms of acquired resistance to paclitaxel have been elucidated. First, mutations of tubulin isotype genes were reported to be a strong determinant of paclitaxel resistance in patients with non-small cell lung cancer [11]. Alterations in tubulin content, expression of tubulin isotype, and polymerization dynamics are considered to be related to resistance to paclitaxel [9]. The second mechanism of acquired resistance to paclitaxel involves the amplification of membrane phosphoglycoproteins that function as drug-efflux pumps [12]. The multidrug-resistant phenotype of tumor cells confers varying degrees of cross-resistance to various agents, including anthracyclines, etoposide, vinca alkaloids, colchicine, and taxanes. Resistance to paclitaxel can be reversed by many types of drugs, including calcium channel blockers, tamoxifen, cyclosporin A, antiarrythmic agents, and principal components of the vehicles used to formulate paclitaxel (cremophor EL) [13]. Several pathways that are involved in apoptosis during development and tumorigenesis, and critical genes in the regulation of these pathways have recently been discovered, e.g., *bcl-2, bcl-x, p53,* and *bax* [14]. Regulation of these apoptosis-related genes may also be involved in the regulation of paclitaxel-induced cytotoxicity and resistance [15].

Toxicities of paclitaxel during cancer therapy

Hypersensitivity reactions

The major hypersensitivity reactions to paclitaxel are dyspnea, bronchospasm, urticaria, and hypotension. These reactions usually occur within 2 to 3 min after the initiation of treatment and are almost noted within the first 10 min. Most of them occur with the first or second

drug administration. These hypersensitivity reactions resolve completely after the paclitaxel infusion is stopped and treatment with histamine receptor antagonists, fluids, and vasopressors is given. Minor hypersensitivity reactions, such as flushing and rashes, have also been noted in as many as 40% of patients. Premedication with corticosteroids and/or H_1, H_2 antagonists decreases the incidence of major hypersensitivity reactions to 1% to 3%.

Hematological toxicity

Neutropenia is the principal hematological toxic effect of paclitaxel. The onset is usually on days 8–10 after treatment and recovery is usually complete by 2 to 3 weeks. The neutropenia is not cumulative, suggesting that paclitaxel does not irreversibly damage immature hematopoietic cells. In most patients, the maximum tolerated dose of paclitaxel without granulocyte colony-stimulating factor is 175–200 mg/m^2 when the drug is administered every 3 weeks. Paclitaxel alone rarely causes thrombocytopenia or anemia.

Neurotoxicity

Peripheral neuropathy characterized by sensory symptoms such as numbness and paresthesia, in a glove-and-stocking-like distribution, is the principal neurotoxic effect of paclitaxel [16]. Severe neurotoxicity precludes a long-term treatment schedule with paclitaxel. The incidence of neurotoxicity has been particularly high in patients who receive paclitaxel as a 3-h infusion, suggesting that peak concentration may be a principal pharmacological determinant. Neurotoxicity seems to occur more frequently and is more serious when paclitaxel is administered in combination with cisplatin. There is no convincing evidence that any specific measure is effective at ameliorating existing manifestations or preventing the development or worsening of the neurotoxicity [17]. Optic nerve disturbances, characterized by scintillating scotomas, may occur in some patients [18].

Muscle toxicity

Transient myalgia, usually noted 2 to 5 days after therapy, is common at paclitaxel doses of 170 mg/m^2 or more, and myopathy is reported with high doses (250 mg/m^2) in combination with cisplatin. Nonsteroidal anti-inflammatory agents are used for palliating and preventing symptoms and narcotics are recommended to be administered prophylactically on days 2 to 5 after treatment in patients who have been symptomatic. Antihistamines have also been reported to be useful in preventing acute myalgia [19].

Cardiac toxicity

The most common cardiovascular symptom with paclitaxel is transient asymptomatic bradycardia, which is noted in 29% of patients [20]. Isolated cardiac bradycardia without hemodynamic effects is not an indication for discontinuing paclitaxel chemotherapy. More important bradyarrythmias and third-degree heart block have also been noted, but the incidence is around 0.1%. Routine cardiac monitoring during paclitaxel therapy is not necessary for most patients, except for those who have the complication of ventricular dysfunction.

Paclitaxel chemotherapy for advanced gastric cancer

Administration of paclitaxel every 3 weeks (3-weekly)

Because complete cure of advanced gastric cancer has not been achieved, the therapeutic goals are the control of disease progression, the relief of symptoms, improvement of quality of life, and the prolongation of survival. Paclitaxel has shown encouraging activity in the treatment of patients with advanced gastric cancer. Historically, paclitaxel has been administered as a bolus infusion every 3 weeks. Monotherapy with paclitaxel in the first-line treatment of advanced disease, as well as in the second-line setting, has produced response rates of approximately 17%-28% [21–24], and considerably longer survival times (median survival time [MST] around 8 months; Table 1) than those seen for other agents with similar response rates. It is the appreciable activity seen in these early phase II studies, along with the lack of cross-resistance to other drugs and the non-overlapping toxicities, that have led researchers to consider further development of the taxanes in combination with existing fluoropyrimidine-platinum regimens in advanced gastric cancer.

In order to improve the results, various combination therapies have been examined in clinical trials. Especially, paclitaxel appears to have a schedule-dependent synergy with platinum compounds, as documented in established human gastric cancer cell lines [25]. This synergy has led to the development of paclitaxel-platinum combination regimens in a number of solid tumors, including gastric cancer. Various phase II studies of 3-weekly paclitaxel-containing combinations in the treatment of patients with advanced gastric cancer are listed in Table 2 [26–41]. Combination regimens of paclitaxel plus platinum, or paclitaxel plus 5-FU, or both, yielded response rates of 32%-65% and MSTs of approximately 11 months (range, 6–14 months) in a first-line treatment setting. With regard to the patients in a setting of more than second-line treatment, the response rates were 22%-28% and median survival

Table 1. Phase II studies of every-3-weeks (3-weekly) paclitaxel monotherapy in advanced and metastatic gastric cancer

Study	Year	Treatment	*n*	Target population	RR (%)	Median progression-free survival (months)	Median survival time (months)
Cascinu et al. [21]	1998	P: 225 mg/m^2 over 3 h	36	Second-line	22	5	8
Ajani et al. [22]	1998	P: 200 mg/m^2 over 3 h	33	First-line	17	6.5	8
Yamada et al. [23]	2001	P: 210 mg/m^2 over 3 h	60	First-line, 34 Prior adjuvant chemotherapy, 6 Second-line, 26	23	5.1	11.3
Yamaguchi et al. [24]	2002	P: 210 mg/m^2 over 3 h	32	First-line, 15 Prior adjuvant chemotherapy, 4 Second-line, 17	28	3	8

n, number of patients; P, paclitaxel; RR, response rate

Table 2. Phase II studies of 3-weekly paclitaxel-containing combinations in advanced and metastatic gastric cancer

Study	Year	Treatment	*n*	Line of treatment	RR (%)	Median progression-free survival (months)	Median survival time (months)
Bokemeyer et al. [26]	1997	P: 175 mg/m^2 F: 2000 mg/m^2 L: 500 mg/m^2	22	First-line	32	8	11
Kim et al. [28]	1999	P: 175 mg/m^2 F: 750 mg/m^2 C: 20 mg/m^2	41	First–line 36 Prior adjuvant chemotherapy 3 Second line 5	51	4 (Median duration of response)	6
Murad et al. [27]	1999	P: 175 mg/m^2 F: 1500 mg/m^2	31	First line	66	9 (Median duration of response)	12
Kollmansberger et al. [29]	2000	P: 175 mg/m^2 F: 2000 mg/m^2 L: 500 mg/m^2 C: 50 mg/m^2	45	First line	51	9	14
Statpoulos et al. [30]	2002	P: 175 mg/m^2 Cb: 5 AUC	47	>Second-line	28	NR	NR
Gadgeel et al. [31]	2003	P: 200 mg/m^2 Cb: AUC 5	27	First-line	33	4.9 (Median duration of response)	7.5
Park et al. [32]	2004	P: 175 mg/m^2 C: 75 mg/m^2	36	First-line	46	4.9	13.8
Chang et al. [33]	2005	P: 200 mg/m^2 Cb: AUC 6	45	>Second-line	22	3.3	7.5
Shin et al. [34]	2005	P: 175 mg/m^2 C: 70 mg/m^2	34	First-line, 24 Second-line, 10	27	6.0	8.9
Lee et al. [35]	2005	P: 145 mg/m^2 C: 60 mg/m^2	39	First-line	44	4.7	12.1
Park et al. [36]	2006	P:175 mg/m^2 F: 500 mg/m^2	38	First-line Prior adjuvant chemotherapy, 11	42	4.3	9.9
Lee et al. [37]	2007	P: 145 mg/m^2 C: 60 mg/m^2	32	Second-line	25	2.9	9.1
Im et al. [38]	2008	P: 175 mg/m^2 F: 1000 mg/m^2 L: 20 mg/m^2	60	First-line 37 Prior adjuvant chemotherapy 13 Second-line 23	32	3.0	14.0
Kang et al. [39]	2008	P: 175 mg/m^2 X: 825 mg/m^2	45	First-line Prior adjuvant chemotherapy 9	49	5.6	11.3
Hwang et al. [40]	2008	P: 175 mg/m^2 C: 75 mg/m^2 F: 750 mg/m^2	45	First-line Prior adjuvant chemotherapy 13	51	6.9	12.7
Jung et al. [41]	2009	P: 135 mg/m^2 C: 30 mg/m^2 F: 1200 mg/m^2 L: 20 mg/m^2	30	NR	46	5.6	9.6

n, number of patients; AUC, area under the concentration-time curve; C, cisplatin; Cb, carboplatin; F, 5-FU; L, folinic acid (LV); P, paclitaxel; X, capecitabine; RR, response rate; NR, not reported

ranged from 6 to 10 months. Although these studies differed with respect to drug regimens and populations treated, the regimens were generally well tolerated, with myelosuppression as the most common toxicity. Other reported toxicities associated with these combination therapies were alopecia, myalgia, mucositis, and neurotoxicity.

The effect of paclitaxel in these combination regimens was obvious, in terms of response rates and MST, compared to paclitaxel monotherapy when the regimens were utilized in a first-line setting. However, in the second-line setting, the combination chemotherapies did not show clear survival benefits compared to the administration of paclitaxel alone.

Weekly administration of paclitaxel

Phase II trials have suggested that weekly paclitaxel may be more effective and less toxic than every-3-week administration for metastatic breast cancer. The Cancer and Leukemia Group B protocol 9840 was initiated to address this question in a phase III trial. The final result was published in 2008, and it was confirmed that weekly paclitaxel administration was superior to an every-3-weeks (3-weekly) paclitaxel schedule for metastatic breast cancer, with a significant increase in response rate and an important advantage in time to progression [42]. Inspired by the results of these studies, studies of weekly paclitaxel, together with various paclitaxel-containing combinations with other chemotherapeutic agents, have been performed for the treatment of advanced gastric cancer.

Monotherapy with weekly paclitaxel in the first-line treatment of advanced disease, as well as in the second-line setting, has produced response rates of approximately 16%-18% and MSTs of around 8 months (Table 3) [43, 44] that were almost identical to the results of 3-weekly administration. However, the quality of life of the patients, and compliance with the study regimens, seemed to be better for weekly administration than for the 3-weekly administration regimen.

Many phase II studies have also been performed to investigate the safety profile and effectiveness of weekly paclitaxel-containing combination therapies for advanced and metastatic gastric cancers (Table 4) [45–61].

Combination therapies with 5-FU+leucovorin or 5-FU were examined in three trials [46, 50, 61]. The addition of either bolus 5-FU (2400–2600 mg/m^2) or 5 days' continuous infusion of 600 mg/m^2 5-FU to weekly paclitaxel at 80 mg/m^2 was proven not to affect the safety of the patients [62]. Response rates ranged from 39% to 41% and median progression-free survival time was more than 3.5 months in all these studies. The MST was also improved, from 8.8 to 11.0 months, suggesting that the combination of weekly paclitaxel with 5-FU is superior to weekly paclitaxel monotherapy in terms of response rate and prognosis.

Weekly paclitaxel combined with cisplatin has also been investigated [51, 52, 54]. Weekly administration of paclitaxel 80 mg/m^2 with weekly cisplatin at 25 mg/m^2 did not show any additional toxicity compared with that of weekly paclitaxel monotherapy [63]. Although the response rates of these regimens varied, from 18% to 41%, the combination of weekly or biweekly paclitaxel with cisplatin showed an improved prognosis of around 11 months.

Combination triplet therapy using paclitaxel, 5-FU, and cisplatin was also studied [45, 53]. Although this type of regimen demonstrated high response rates, of around 50%, median survival was around 11 months, and was not very much improved compared to that with doublet paclitaxel-5-FU regimens or paclitaxel-cisplatin regimens. A new phase II trial is now under way, according to the recommended dose of weekly paclitaxel 80 mg/m^2, cisplatin 25 mg/m^2, and 5-FU 600 mg/m^2, that was suggested by a high response rate of 83% in a phase I trial [64].

With regard to oral chemotherapeutic agents, weekly paclitaxel combined with oral UFT (uracil, tegafur) plus leucovorin showed a response rate of 50% and MST of 9.8 months [48]. Studies of combinations with oral S-1 (tegafur, gimeracil, oteracil) have also been performed [47, 49, 55–59]. In these trials, response rates ranged from 40% to 65% and MSTs ranged from 8.9 to 15.5 months. Weekly administration of 40–60 mg/m^2 paclitaxel combined with 80 mg/m^2 of S-1 for 14 days in a 4-week cycle [65] seemed to have superior benefit in terms of prognosis (median, 13.85 months) compared to

Table 3. Phase II studies of weekly paclitaxel monotherapy in advanced and metastatic gastric cancer

Study	Year	Treatment	*n*	Line of treatment	RR (%)	Median progression-free survival (months)	Median survival time (months)
Kodera et al. [43]	2007	P: 80 mg/m^2/week > 3/4 weeks	45	Second-line	16	2.6	7.8
Emi et al. [44]	2008	P: 80 mg/m^2/week > 3/4 weeks	68	First-line	17.6	3.2	7.3

n, number of patients

Table 4. Phase II studies of weekly (w) or biweekly (2 w) paclitaxel-containing combinations for advanced and metastatic gastric cancer

Study	Year	Treatment	*n*	Line of treatment	RR (%)	Median progression-free survival (months)	Median survival time (months)
Honecker et al. [45]	2002	P: 80 mg/m^2 (w) F: 2000 mg/m^2 L: 500 mg/m^2 C: 50 mg/m^2	29	First-line	48	8	11
Yeh et al. [46]	2005	P: 80 mg/m^2 (w) F: 2600 mg/m^2 L: 300 mg/m^2	30	First-line Prior adjuvant chemotherapy 2	41	6	10
Mochiki et al. [47]	2006	P: 60 mg/m^2 (w) S: 80 mg/m^2	24	First-line Prior adjuvant chemotherapy 4	54	9.5	15.5
Chao et al. [48]	2006	P: 100 mg/m^2 (w) U:300 mg/m^2 L: 90 mg/m^2	55	First-line Prior adjuvant chemotherapy 2	50	4.4	9.8
Kawabata et al. [49]	2007	P: 50 mg/m^2 (w) S: 80 mg/m^2	18	First-line	65	9.1	13.8
Ninomiya et al. [50]	2007	P: 80 mg/m^2 (w) F: 600 mg/m^2	57	First-line 32 Second-line 25	39	5.4	11
Kim et al. [51]	2007	P: 140 mg/m^2 (2 w) C: 30 mg/m^2	50	First-line 35 Second-line 15	18	2.9	11.1
Kim et al. [52]	2007	P: 100 mg/m^2 (w) C: 35 mg/m^2	52	First-line	36.5	6.0	10.8
Gu et al. [53]	2008	P: 60 mg/m^2 (w) F: 500 mg/m^2 C: 75 mg/m^2	46	First-line	50	5.6	10.8
Nagata et al. [54]	2008	P: 80 mg/m^2 (w) C: 25 mg/m^2	49	First-line 25 Second-line 24	41	5.5	10.9
Nakajo et al. [55]	2008	P: 120 mg/m^2 (2 w) S: 80 mg/m^2	39	First-line Prior adjuvant chemotherapy 4	45	4.1	8.5
Inada et al. [56]	2009	P: 50 mg/m^2(w) S: 80 mg/m^2	22	First-line Prior adjuvant chemotherapy 1	55	4.7	9.5
Lee et al. [57]	2008	P: 70 mg/m^2 (w) S: 70 mg/m^2	56	First-line Prior adjuvant chemotherapy 9	40	6.6	12.1
Narahara et al. [58]	2008	P: 40 mg/m^2 (w) S: 80 mg/m^2	29	First-line 24 Second-line 5	48	NR	13.9
Ueda et al. [59]	2006	P: 50 mg/m^2 (w) S: 80 mg/m^2	54	First-line	46	6.1	14.5
Takiuchi et al. [60]	2008	P: 80 mg/m^2(w) 5'DFUR: 600 mg/m^2	35	Second-line S-1-refractory	18	4.0	10.9
Lee et al. [61]	2009	P: 75 mg/m^2 (2 w) F: 2400 mg/m^2 L: 40 mg	30	First-line Prior adjuvant chemotherapy 5	40	3.9	8.8

n, number of patients; C, cisplatin; U, UFT; F, 5-FU; L, folinic acid (LV); P, paclitaxel; S, S-1; 5'DFUR, doxifluridine; RR, response rate; NR, not reported

biweekly administration of paclitaxel plus S-1. Because the background of patients who are eligible for a combination of paclitaxel with oral agents is assumed to be better, because of their possibility of oral intake, it is not necessarily surprising that paclitaxel plus S-1 showed the most marked improvement in prognosis in patients with advanced and metastatic gastric cancer.

Paclitaxel chemotherapy for peritoneal dissemination and ascites

Peritoneal dissemination and ascites seem to be one of the most horrible and wretched features of gastric cancer. Only eight reports were found to have evaluated the effect of paclitaxel for peritoneal carcinomatosis, a common pattern of failure among gastric cancer patients. In case studies, complete disappearance of gastric ascites was reported to have been brought