and the National Institutes of Health trial registry (ClinicalTrials.gov). Trials were eligible if they were randomized, closed to patient accrual before 2004, and compared any adjuvant therapy after curative resection with surgery alone.

Data and Outcomes

The following data were requested for all individual patients included in all the trials: center, randomization date, treatment allocated by randomization, date of last follow-up or death, survival status, cause of death (if applicable), relapse status, and type and date of relapse if any. OS was defined as the time from randomization to all-cause death or the date of the last follow-up used for censoring. DFS was defined as the time to relapse, second cancer, or all-cause death, whichever came first. Detailed information on the type of relapse was not always available. All data were centrally reanalyzed and checked for inconsistencies. In particular, diagnostic tools for randomization quality were systematically applied (6).

Statistical Methods

Forest plots were used to display the hazard ratios (HRs) for overall and individual trials, which were then used for the evaluation of surrogacy of DFS for OS (labeled "training trials" in Figure 1) and for external validation trials (labeled "validation literature data" and "validation trials IPD" in Figure 1). The hazard ratios compared the hazard of an event in patients treated with adjuvant chemotherapy with the hazard in patients treated with surgery alone.

We used the Spearman rank correlation coefficient between DFS and OS to assess surrogacy at the individual level and the coefficient of determination between the natural logarithm of the hazard ratios for DFS and OS to assess surrogacy at the trial level (7,9). At the individual level, the association between the distribution of the true endpoint (OS) and the surrogate (DFS) was evaluated using a bivariable model based on the Plackett copula combined with trial-specific Weibull models for DFS and OS (10,11). The association between the estimates of treatment effects obtained using the bivariable model was used to assess surrogacy at the trial level. A good surrogate was considered to provide a reliable prediction of the treatment effect on the true endpoint (eg, the hazard ratio for OS) from the treatment effect on the surrogate (eg, the hazard ratio for DFS). It should be noted that estimates of the hazard ratios based on the bivariable model might differ from the crude estimates shown in the forest plot.

To quantify the association between the natural logarithm of the hazard ratios for OS and DFS, we used a linear regression model that accounted for the uncertainty about the estimated effects by using an error-in-variables linear regression model. The strength of the association was assessed by using the coefficient of determination R^2 (or explained variation). This approach has been previously used for resectable colorectal and metastatic breast cancers (7,12).

Sensitivity Analyses

To assess the typical trial conditions, we performed a sensitivity analysis by studying the association between the treatment effects on OS at 5 years and DFS at different time points (2 years, 3 years, and 4 years), while censoring all events occurring after these time points. Because only durations (OS and DFS) and not dates were provided for two studies, the same individual follow-up was used for all patients, irrespective of their actual accrual date. In this analysis, the number of observed events is considerably lower than that in the analysis of patients followed-up to a common administrative censoring date. In the latter case, analysis takes place 2, 3, or 4 years after the accrual of the last patients. Therefore, the first accrued patient may have much longer follow-up.

External Validation

To assess the external validity of our results, we used 4 trials for which we did not receive IPD from the principal investigators [no reply or refusal to share the data (13, 14) or data lost (15)] and the large-scale CLASSIC trial for which only interim analysis was available at the time of the surrogate analysis (2). We extracted DFS and OS from the summary statistics published for these trials (16). We also used the IPD from a large trial investigating the effect of adjuvant treatment with S1 (TS-1, Taiho Pharmaceutical Company of Bejing Ltd, Bejing, China) vs surgery alone (5) and from a trial studying the benefit of postoperative chemoradiation vs surgery (4).

Surrogate Threshold Effect

On the basis of a linear regression model adjusted for estimation error in observed treatment effects, we calculated the surrogate threshold effect (STE), defined as the minimum treatment effect on DFS necessary to predict a nonzero effect on OS (17). A future trial would require the upper limit of the confidence interval for the estimated hazard ratio for DFS to fall below the STE to predict a nonzero effect on OS.

All analyses were performed on an intention-to-treat basis. Confidence intervals (CI) were calculated for a two-sided probability coverage of 95%. All analyses were performed using SAS software v9.3 (SAS Institute Inc., Cary, NC) except for the graphical displays (double forest plots were plotted using a set of R functions developed at the International Drug Development Institute [Louvain-la-Neuve, Belgium], whereas other figures were prepared using STATA v12 [StataCorp LP, College Station, TX]).

Results

Data were obtained on 3371 patients from the 14 eligible randomized trials with documented OS and DFS (18–30). Nonmissing data on both endpoints were available for 3288 patients, of whom 1763 had events related to DFS and 1705 died during follow-up. Detailed information about treatment regimens and median follow-up studies is provided in Supplementary Table 1 (available online). Figure 1 shows a forest plot of the treatment effects on OS and DFS for all trials. Figure 2 shows the overall Kaplan–Meier curves for DFS and OS. Overall and at the trial level, the effect of any adjuvant chemotherapy on DFS appeared close to the effect on OS ($HR_{OS} = 0.86$; $HR_{DFS} = 0.82$).

Individual- and Trial-Level Association

The individual-level association, as measured by the Spearman rank correlation coefficient, was as high as 0.974 (95% CI = 0.971 to 0.976), indicating a very strong correlation between DFS and OS for a given patient.

A high correlation was noted between log HR_{OS} and log HR_{DFS} (Figure 3). The coefficient of determination, R^2, for the estimated treatment effects was 0.964 (95% CI = 0.926 to 1.000) and 1.000

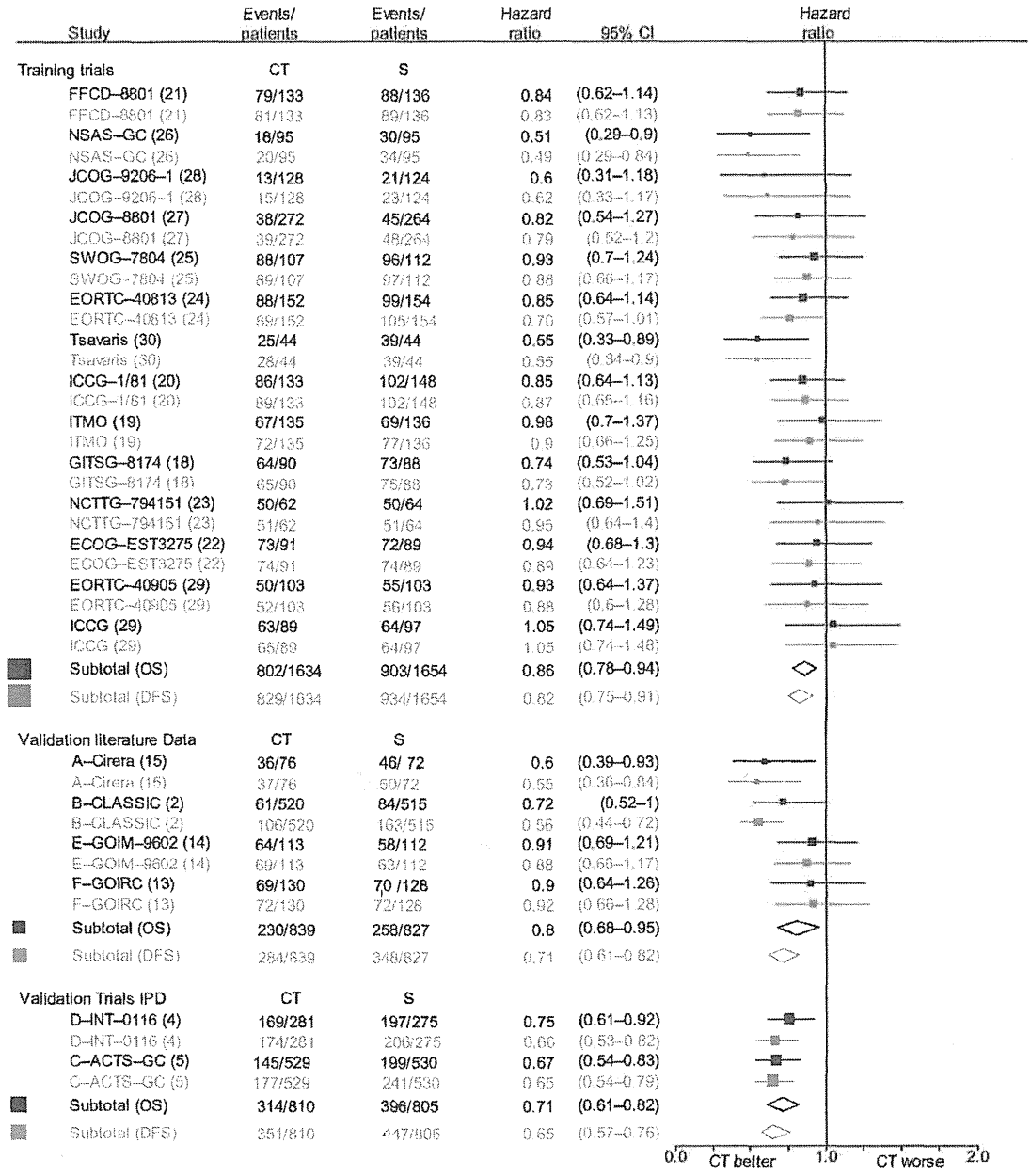


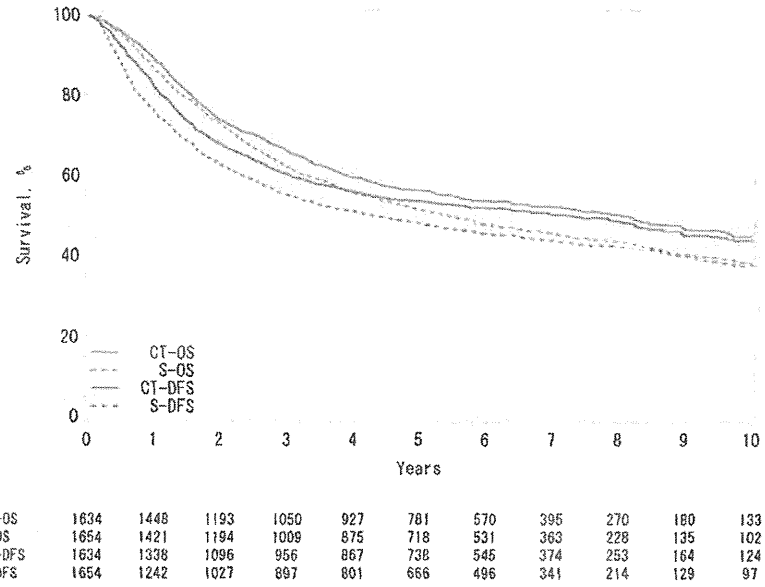
Figure 1. Forest plot of treatment effects (hazard ratios) on disease-free survival (DFS) and on overall survival (OS). The **first row** for each trial shows the result for OS, and the **second row** shows the result of DFS. The **squares** and **diamonds** represent the point estimates and pooled estimates, respectively. **Sizes of the symbols** represent the number of events. The **horizontal error bars** show the 95% confidence interval (CI) of each hazard ratio. CT = adjuvant chemotherapy; IPD = individual patient data; S = surgery alone.

(95% CI = 0.999 to 1.000) before and after adjusting for the estimation error, respectively. Notably, however, because the estimated R^2 value was very close to the upper limit of 1, the obtained numerical results need to be interpreted with caution, as they can be easily influenced by numerical errors.

The linear regression model adjusted for estimation errors was as follows:

$$\ln(\mathrm{HR}_{\mathrm{OS}}) = 0.047 + 1.239 \times \ln(\mathrm{HR}_{\mathrm{DFS}}).$$

In the equation, $\ln(\mathrm{HR}_{\mathrm{OS}})$ and $\ln(\mathrm{HR}_{\mathrm{DFS}})$ denote the natural log transformation of the hazard ratio for each endpoint. Standard errors were 0.023 and 0.151 for the intercept and slope, respectively. This is shown as a straight line on Figure 3, where the x-axis represents the treatment effect on DFS and the y-axis represents the treatment effect on OS. Each trial is represented by a bubble of a size proportional to the trial sample size. The 95% prediction limits indicate the range of effect on OS that can be expected for a given effect on DFS.



CT-OS	1634	1448	1193	1050	927	781	570	395	270	180	133
S-OS	1654	1421	1194	1009	875	718	531	363	228	135	102
CT-DFS	1634	1338	1096	956	867	738	545	374	253	164	124
S-DFS	1654	1242	1027	897	801	666	496	341	214	129	97

Figure 2. Disease-free survival (DFS) and overall survival (OS) Kaplan–Meier survival curves truncated at 10 years. The number of patients at risk in each group is given below the graph. CT = adjuvant chemotherapy; S = surgery alone.

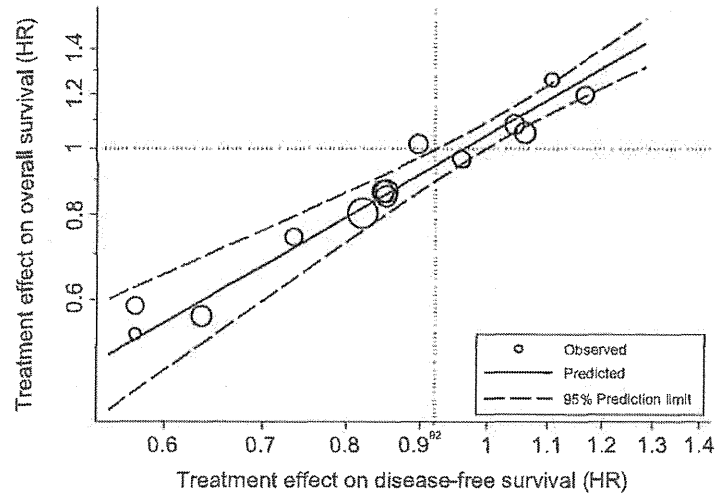


Figure 3. Trial-level association between treatment effects. Log scale was used for the x-axis and y-axis. The **horizontal line (dots)** corresponds to the hazard ratio (HR) on overall survival (OS) of 1—that is, the absence of effect on the OS. The **vertical line (dots)** crosses the upper boundary of the 95% prediction limit at the point hazard ratio on OS equal to 1. This indicates the surrogate threshold effect.

Considering the high correlation at both the individual and the trial levels, we also computed the STE based on the adjusted regression model. The STE is defined as the intersection of the upper prediction limit, with the horizontal line representing a hazard ratio of 1 for OS (null hypothesis). The STE was equal to 0.92; hence, in a future trial using similar treatment modalities, as in our set of trials, a hazard ratio for DFS less than 0.92 would predict with 95% probability a hazard ratio for OS less than 1.

Sensitivity Analyses

Table 1 shows the association between OS and DFS measured at different time points, ranging from 2 to 4 years after randomization. For this analysis, we report the number of the events available for both OS at 5 years and DFS at 2, 3, and 4 years. Note that at 2 and 3 years, the number of events for DFS was actually lower than that for OS at 5 years, which resulted in wider confidence intervals. Therefore, for this sensitivity analysis, we present values unadjusted for the estimation error.

External Validation

Table 2 and Figure 4 show the results of the external validation using summary data (2,13–15) and IPD (4,5) for six trials. The table displays the observed hazard ratios for OS and DFS with 95% confidence intervals and the hazard ratio for OS predicted from the model of Figure 3 with the 95% predictive prediction intervals. Notably, the 95% confidence interval quantifies the uncertainty of the estimates of

Table 1. Correlation between survival endpoints and surrogacy measures quantification based on the individual patient data from 14 randomized controlled trials*

Summary measures	2-year DFS/5-year OS	3-year DFS/5-year OS	4-year DFS/5-year OS	All
Events	1135/1489	1379/1489	1511/1489	1763/1705
Rho† (95% CI)	0.949 (0.943 to 0.955)	0.953 (0.948 to 0.958)	0.957 (0.952 to 0.961)	0.974 (0.971 to 0.976)
Unadjusted R^2 (95% CI)‡	0.776 (0.569 to 0.983)	0.866 (0.736 to 0.997)	0.918 (0.835 to 1.000)	0.964 (0.926 to 1.001)
Unadjusted regression§	0.083 + 0.886 × TE	0.069 + 1.004 × TE	0.061 + 1.092 × TE	0.040 + 1.155 × TE
Adjusted regression‖	0.565 + 3.957 × TE	0.213 + 2.308 × TE	0.109 + 1.691 × TE	0.047 + 1.239 × TE
STE (HR)	Undefined	Undefined	0.77	0.92

* CI = confidence interval; DFS = disease-free survival; HR = hazard ratio; OS = overall survival; STE = surrogate threshold effect calculated on adjusted regression; TE = treatment effect on disease-free survival in the prediction model for treatment effect on overall survival.

† Rho represents the Spearman rank correlation coefficient between disease-free survival and overall survival.

‡ R^2 represents the coefficient of determination between treatment effect on disease-free survival and overall survival.

§ Unadjusted regression represents the linear regression prediction models for the treatment effect on overall survival from treatment effect on disease-free survival, unadjusted for the presence of estimation error in the treatment effects.

‖ Adjusted regression represents the linear regression prediction models for the treatment effect on overall survival from treatment effect on disease-free survival, adjusted for the presence of estimation error in the treatment effects.

Table 2. Observed and predicted treatment effect on overall survival based on the observed treatment effect on disease-free survival*

Trial label	Validation trials (reference)	Type of data	Observed HR_{DFS} (95% CI)	Observed HR_{OS} (95% CI)	Predicted HR_{OS} (95% PI)
A	Cirera et al. (15)	Published	0.55 (0.36 to 0.85)	0.60 (0.39 to 0.93)	0.50 (0.28 to 0.87)
B	CLASSIC (2)	Published	0.56 (0.44 to 0.72)	0.72 (0.52 to 1.00)	0.51 (0.36 to 0.73)
C	ACTS-GC (5)	IPD	0.65 (0.54 to 0.79)	0.67 (0.54 to 0.83)	0.61 (0.47 to 0.81)
D	INT-1018 (4)	IPD	0.66 (0.53 to 0.82)	0.75 (0.61 to 0.92)	0.63 (0.46 to 0.84)
E	GOIM- 9602 (14)	Published	0.88 (0.66 to 1.17)	0.91 (0.69 to 1.21)	0.89 (0.62 to 1.28)
F	GOIRC (13)	Published	0.92 (0.66 to 1.27)	0.90 (0.64 to 1.26)	0.94 (0.63 to 1.42)

* CI = confidence interval; DFS = disease-free survival; HR = hazard ratio; IPD = individual patient data; OS = overall survival; PI = prediction interval.

the hazard ratios on the basis of the events observed in each validation trial, whereas the prediction interval quantifies the uncertainty of the predicted hazard ratio for OS (without the information of OS) as a function of the observed hazard ratio for DFS. The difference between the confidence interval and the prediction interval is explained in further detail in the Supplementary Methods (available online).

Excellent agreement was noted between the observed and predicted hazard ratios for OS for two (13,14) (labeled E and F in Figure 3 and Table 2) of the four trials for which only summary data were available. The hazard ratio for OS predicted from the estimated hazard ratio for DFS after a median 5-year follow-up was lower than the observed value but still within the prediction interval for the two validation trials (2,15) (labeled A and B in Figure 3 and Table 2) for which IPD could not be obtained.

For the large Japanese trial investigating the effect of adjuvant treatment with S1 (5) (labeled C in Table 2 and Figure 4), the observed and predicted hazard ratios for OS were in reasonable agreement. For the trial (4) (labeled D in Table 2 and Figure 4) investigating the efficacy of adjuvant chemoradiation, the predicted hazard ratio for OS was also lower than the observed hazard ratio for OS, although the latter still fell within the 95% prediction interval.

Discussion

Our results show a very tight individual-level association between DFS and OS (Spearman rank correlation coefficient = 0.974; 95% CI = 0.971 to 0.976), indicating that in individual patients, DFS is highly predictive of OS. The strong correlation between DFS and OS can be partly attributed to the short time from relapse to death in gastric cancer (median of <12 months across all the included trials). Further, 16% of all the analyzed patients died without documented relapse and, therefore, had the same DFS and OS.

We also found a very high trial-level association between the effects of adjuvant chemotherapy on DFS and on OS, with R^2 being almost 1, which indicates that almost all of the variability in the treatment effects on OS can be explained by the treatment effects on DFS (Figures 1 and 3). We constructed the prediction limits around the regression line, which accounts for the fact that the hazard ratios of DFS and OS were estimated with errors. STE was found to be 0.92, thereby implying that a treatment producing an 8% or greater hazard reduction for recurrence can be expected to produce a statistically significant hazard reduction for death. STE also reflects the expected dilution of the treatment effect on OS as compared with the effect on DFS. With a reduced duration of follow-up, a stronger effect on DFS was required to predict a statistically significant benefit over OS. This is partly because of the loss of events due to the shorter period of observation. At 4 years, a hazard ratio of 0.74 was required. In case follow-up was truncated at 2 or 3 years, STE could not be estimated. A fixed follow-up period was used in this sensitivity analysis because the date of randomization was not available for all studies. In trials with an administrative censoring date common to all patients, more events would be available at the intermediate time point, resulting in more precise estimates. We also did not collect information on the treatment administered after recurrence. However, the median OS of the patients with advanced gastric cancer treated with chemotherapy was 8.7 months in the GASTRIC

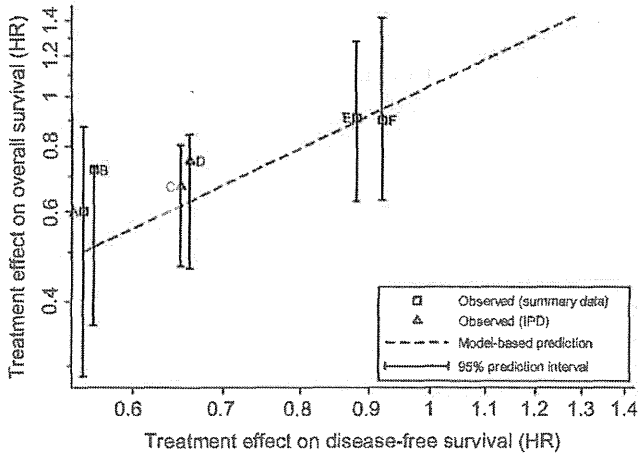


Figure 4. Observed treatment effect on disease-free survival vs predicted treatment effect on overall survival in validation trials. The error bars represent 95% prediction intervals. Log scale was used for the x-axis and y-axis. For the trial labels, A represents Cirera et al. (15), B represents CLASSIC (2), C represents ACTS-GC (5), D represents INT-1018 (4), E represents GOIM- 9602 (14), and F represents GOIRC (13), as shown in Table 2. HR = hazard ratio; IPD = individual patient data.

database (31), and the impact of chemotherapy on OS after relapse was not much greater than that of adjuvant chemotherapy. Thus the fact that some patients received chemotherapy on relapse is not expected to have a major impact on our findings.

In a future trial testing a new treatment for gastric cancer, interest would focus on predicting the effects on OS at some time point (eg, 5 years), having observed the effects on DFS at an earlier time point. The results presented in Table 1 suggest that the measurement of DFS at 2 years may be too early to enable an accurate prediction of OS at 5 years. With very early time points, only few DFS events are available, which may result in imprecise predictions; on the other hand, very late time points are less useful because they are closer to the evaluation of the final endpoint. Making analysis at 3 or 4 years would probably reduce the overall duration by about 15% to 30% if the accrual was short enough.

Do the present results justify the use of DFS as a surrogate for OS in resectable gastric cancer? A large proportion of relapses occurred before 3 years, and we found a strong correlation between the endpoints, both at the individual and trial levels. Similar results have led to the adoption of the 3-year DFS as a surrogate for 5-year OS in evaluating new treatments for resectable colon cancer (32). Our results are based on fewer trials and smaller sample sizes, but they include a broader range of treatment options. One may be interested in whether DFS would be a surrogate for OS for all studies independent of geography because it is well known that there exists a large heterogeneity about the prognosis between Asian and non-Asian patients. In spite of the prognostic heterogeneity between continents, there was no statistically significant heterogeneity about the treatment effects on OS and on DFS between Asia and non-Asia trials (6). In addition, the relationship between the hazard ratio for OS and the hazard ratio for DFS was also clearly consistent throughout all trials. Therefore, we believe the use of DFS as a surrogate would be independent of the geography. Moreover, we were able to use the published results of trials not included in our meta-analysis, as well as the IPD from two large trials, as two independent validation sets. The hazard ratios fell within the prediction intervals for all six available trials (Table 2). The results of the four trials with literature data only should, however, be interpreted with caution because they are based on extracted summary statistics. The relationship between the treatment effect on DFS and that on OS, as established in trials comparing adjuvant chemotherapy with surgery alone, seems to be verified for the chemoradiation trial, which implies that our results might be applicable to more general adjuvant treatments with a curative intent.

One should keep in mind the following limitations. Numerical computational issues may have slightly biased correlation estimates. Subgroup analysis based on the baseline variables, including continents, could not be performed because of the small number of trials. Similar to the case with interim analyses, follow-up after the analysis of the surrogate endpoint (DFS) is necessary to determine the OS, safety, and post-relapse outcomes as well as to document the possible impact of postrelapse treatments for advanced diseases. An important consideration is that we only investigated cytotoxic agents, and future trials investigating agents with different mechanisms of actions, such as target therapy, will require separate validation of the surrogacy relation before it is applied routinely.

In conclusion, the treatment effect on OS is largely predictable according to that on DFS; therefore, DFS can be used as a primary endpoint for further clinical trials of adjuvant chemotherapies, thus reducing the duration by 15% to 30% and the cost, depending on the planned follow-up, of these large-scale randomized trials.

References

1. Ferlay J, Shin H-R, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer.* 2010;127(12):2893–2917.
2. Bang Y-J, Kim Y-W, Yang H-K, et al. Adjuvant capecitabine and oxaliplatin for gastric cancer after D2 gastrectomy (CLASSIC): a phase 3 open-label, randomised controlled trial. *Lancet.* 2012;379(9813):315–321.
3. Cunningham D, Allum WH, Stenning SP, et al. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. *N Engl J Med.* 2006;355(1):11–20.
4. Macdonald JS, Smalley SR, Benedetti J, et al. Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction. *N Engl J Med.* 2001;345(10):725–730.
5. Sasako M, Sakuramoto S, Katai H, et al. Five-year outcomes of a randomized phase III trial comparing adjuvant chemotherapy with S-1 versus surgery alone in stage II or III gastric cancer. *J Clin Oncol.* 2011;29(33):4387–4393.
6. The GASTRIC group. Benefit of adjuvant chemotherapy for resectable gastric cancer: a meta-analysis. *JAMA.* 2010;303(17):1729–1737.
7. Buyse M, Burzykowski T, Michiels S, Carroll K. Individual- and trial-level surrogacy in colorectal cancer. *Stat Methods Med Res.* 2008;17(5):467–475.
8. Mauguen A, Pignon J-P, Burdett S, et al. Surrogate endpoints for overall survival in chemotherapy and radiotherapy trials in operable and locally advanced lung cancer: a re-analysis of meta-analyses of individual patients' data. *Lancet Oncol.* 2013;14(7):619–626.
9. Buyse M, Molenberghs G, Burzykowski T, Renard D, Geys H. The validation of surrogate endpoints in meta-analyses of randomized experiments. *Biostatistics.* 2000;1(1):49–67.
10. Burzykowski T, Molenberghs G, Buyse M. *The Evaluation of Surrogate Endpoints.* New York: Springer; 2006
11. Burzykowski T, Molenberghs G, Buyse M, Geys H, Renard D. Validation of surrogate end points in multiple randomized clinical trials with failure time end points. *J R Stat Soc Ser C Appl Statist.* 2001;50(4):405–422.
12. Burzykowski T, Buyse M, Piccart-Gebhart MJ, et al. Evaluation of tumor response, disease control, progression-free survival, and time to

progression as potential surrogate end points in metastatic breast cancer. *J Clin Oncol.* 2008;26(12):1987–1992.
13. Di Costanzo F, Gasperoni S, Manzione L, et al. Adjuvant chemotherapy in completely resected gastric cancer: a randomized phase III trial conducted by GOIRC. *J Natl Cancer Inst.* 2008;100(6):388–398.
14. De Vita F, Giuliani F, Orditura M, et al. Adjuvant chemotherapy with epirubicin, leucovorin, 5-fluorouracil and etoposide regimen in resected gastric cancer patients: a randomized phase III trial by the Gruppo Oncologico Italia Meridionale (GOIM 9602 Study). *Ann Oncol.* 2007;18(8):1354–1358.
15. Cirera L, Balil A, Batiste-Alentorn E, et al. Randomized clinical trial of adjuvant mitomycin plus tegafur in patients with resected stage III gastric cancer. *J Clin Oncol.* 1999;17(12):3810–3815.
16. Parmar MK, Torri V, Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. *Statist Med.* 1998;17(24):2815–2834.
17. Burzykowski T, Buyse M. Surrogate threshold effect: an alternative measure for meta-analytic surrogate endpoint validation. *Pharm Statist.* 2006;5(3):173–186.
18. The Gastrointestinal Tumor Study Group. Controlled trial of adjuvant chemotherapy following curative resection for gastric cancer. The Gastrointestinal Tumor Study Group. *Cancer.* 1982;49(6):1116–1122.
19. Bajetta E, Buzzoni R, Mariani L, et al. Adjuvant chemotherapy in gastric cancer: 5-year results of a randomised study by the Italian Trials in Medical Oncology (ITMO) Group. *Ann Oncol.* 2002;13(2):299–307.
20. Coombes RC, Schein PS, Chilvers CE, et al. A randomized trial comparing adjuvant fluorouracil, doxorubicin, and mitomycin with no treatment in operable gastric cancer. International Collaborative Cancer Group. *J Clin Oncol.* 1990;8(8):1362–1369.
21. Bouché O, Ychou M, Burtin P, et al. Adjuvant chemotherapy with 5-fluorouracil and cisplatin compared with surgery alone for gastric cancer: 7-year results of the FFCD randomized phase III trial (8801). *Ann Oncol.* 2005;16(9):1488–1497.
22. Engstrom PF, MacIntyre JM, Douglass HO, Muggia F, Mittelman A. Combination chemotherapy of advanced colorectal cancer utilizing 5-fluorouracil, semustine, dacarbazine, vincristine, and hydroxyurea: a phase III trial by the Eastern Cooperative Oncology Group (EST: 4275). *Cancer.* 1982;49(8):1555–1560.
23. Krook JE, O'Connell MJ, Wieand HS, et al. A prospective, randomized evaluation of intensive-course 5-fluorouracil plus doxorubicin as surgical adjuvant chemotherapy for resected gastric cancer. *Cancer.* 1991;67(10):2454–2458.
24. Lise M, Nitti D, Marchet A, et al. Final results of a phase III clinical trial of adjuvant chemotherapy with the modified fluorouracil, doxorubicin, and mitomycin regimen in resectable gastric cancer. *J Clin Oncol.* 1995;13(11):2757–2763.
25. Macdonald JS, Fleming TR, Peterson RE, et al. Adjuvant chemotherapy with 5-FU, adriamycin, and mitomycin-C (FAM) versus surgery alone for patients with locally advanced gastric adenocarcinoma: a Southwest Oncology Group study. *Ann Surg Oncol.* 1995;2(6):488–494.
26. Nakajima T, Kinoshita T, Nashimoto A, et al. Randomized controlled trial of adjuvant uracil-tegafur versus surgery alone for serosa-negative, locally advanced gastric cancer. *Br J Surg.* 2007;94(12):1468–1476.
27. Nakajima T, Nashimoto A, Kitamura M, et al. Adjuvant mitomycin and fluorouracil followed by oral uracil plus tegafur in serosa-negative gastric cancer: a randomised trial. Gastric Cancer Surgical Study Group. *Lancet.* 1999;354(9175):273–277.
28. Nashimoto A, Nakajima T, Furukawa H, et al. Randomized trial of adjuvant chemotherapy with mitomycin, fluorouracil, and cytosine arabinoside followed by oral fluorouracil in serosa-negative gastric cancer: Japan Clinical Oncology Group 9206-1. *J Clin Oncol.* 2003;21(12):2282–2287.
29. Nitti D, Wils J, Dos Santos JG, et al. Randomized phase III trials of adjuvant FAMTX or FEMTX compared with surgery alone in resected gastric cancer. A combined analysis of the EORTC GI Group and the ICCG. *Ann Oncol.* 2006;17(2):262–269.
30. Tsavaris NB, Tentas K, Kosmidis P, et al. 5-Fluorouracil, epirubicin, and mitomycin C versus 5-fluorouracil, epirubicin, mitomycin C, and leucovorin in advanced gastric carcinoma. A randomized trial. *Am J Clin Oncol.* 1996;19(5):517–521.
31. The GASTRIC Group. Role of chemotherapy for advanced/recurrent gastric cancer: an individual-patient-data meta-analysis. *Eur J Cancer.* 2013;49(7):1565–1577.
32. Sargent DJ, Wieand HS, Haller DG, et al. Disease-free survival versus overall survival as a primary end point for adjuvant colon cancer studies: individual patient data from 20,898 patients on 18 randomized trials. *J Clin Oncol.* 2005;23(34):8664–8670.

Funding

This work was partially supported by the French Institut National du Cancer (grant PHRC GASTRIC); the Clinical Research Support Unit; and the Epidemiological and Clinical Research Information Network. A meeting was supported financially by unrestricted grants from GlaxoSmithKline. The funders were not present at the meeting, were not involved in the analysis of the data, and did not comment on the present paper.

Notes

K. Oba and X. Paoletti are co–first authors; M. Buyse and T. Burzykowski are co–last authors.

The project was initiated under the auspice of the French National Cancer Institute (INCa), which served as the sponsor. The INCa centralized all the databases and provided administrative and data management support. The sponsor has not participated in the analysis and interpretation of the data, which were solely the responsibility of the writing committee. The sponsor played no part in the preparation, review, or approval of the manuscript. The conclusions may not reflect the recommendations of the INCa.

The GASTRIC Group: Secretariat: Marc Buyse, Stefan Michiels, Koji Oba, Xavier Paoletti, Philippe Rougier, Seiichiro Yamamoto, and Kenichi Nakamura. Steering Committee: Yung-Jue Bang (Seoul National University College of Medicine, Seoul, Korea); Harry Bleiberg (Brussels, Belgium); Tomasz Burzykowski (Hasselt University, Diepenbeek, Belgium); Marc Buyse (International Drug Development Institute, Louvain-la-Neuve, Belgium); Catherine Delbaldo (Hôpital Louis Mourier, Colombes, France); Stefan Michiels (Institut Gustave-Roussy, Villejuif, France); Satoshi Morita (Yokohama City University, Yokohama, Japan); Koji Oba (Hokkaido University Hospital, Sapporo, Japan); Yasuo Ohashi (University of Tokyo, Tokyo, Japan); Xavier Paoletti (Institut Curie, Paris, France); Jean-Pierre Pignon (Institut Gustave-Roussy, Villejuif, France); Philippe Rougier (University Hospital Europeen Georges Pompidou, Paris, France); Junichi Sakamoto (Tokai Central Hospital, Kakumuhara, Japan); Daniel Sargent (Mayo Clinic, Rochester, Minnesota); Mitsuru Sasako (Hyogo College of Medicine, Nishinomiya, Japan); and Eric Van Cutsem (University Hospital Gasthuisberg, Leuven, Belgium). Collaborators who supplied individual patient data: Steven Alberts, Mayo Clinic, Rochester, Minnesota; Emilio Bajetta, Department of Medical Oncology, IRCCS Fondazione Istituto Nazionale dei Tumori, Milan, Italy; Jacqueline Benedetti, SWOG Statistical Center, Seattle, Washington; Franck Bonnetain, FFCD, Dijon, France; Olivier Bouche, Department of Gastro-Intestinal Oncology, University Hospital Robert Debré, Reims, France; Paul Catalano, Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, and Department of Biostatistics, Harvard School of Public Health, Boston, Massachusetts; R. Charles Coombes, Medical Oncology Unit, Charing Cross Hospital, London, UK; Maria Di Bartolomeo, Department of Medical Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy; Juan J. Grau, Department of Oncology, Institut Clinic de Malalties Hemato-Oncologiques of Hospital Clinic, University of Barcelona, Spain; James E. Krook, Mayo Clinic, Rochester, Minnesota; Florian Lordick, University Clinic Leipzig; University Cancer Center, Leipzig, Germany; Mario Lise, Department of Oncological and Surgical Sciences, University of Padua, Padua, Italy; John S. Mac Donald, St. Vincent's Cancer Center, New York, New York; Pierre Michel, Department of Gastro-Intestinal Oncology, Charles-Nicolle University Hospital, Rouen, France; Toshifusa Nakajima, Cancer Institute Hospital, Tokyo, Japan; Atsushi Nashimoto, Niigata Cancer Center Hospital, Niigata, Japan; Garth D. Nelson, Mayo Clinic, Rochester, Minnesota; Donato Nitti, Department of Oncological and Surgical Sciences, University of Padua, Italy; Jan Kulig, Piotr Kolodziejczak, and Tadeusz Popiela, 1st Chair of General and GI Surgery, Jagiellonian University Medical College, Krakow, Poland; Philippe Rougier, University Hospital Ambroise Paré, Boulogne,

France; Nicolas Tsavaris, Laiko University Hospital, Athens, Greece; and Mitsuru Sasako, Hyogo College of Medicine, Nishinomiya, Japan.

The GASTRIC Group thanks all the patients who took part in the trials and contributed to this research. The meta-analysis would not have been possible without the active participation of the collaborating institutions that provided trial data: Eastern Cooperative Oncology Group, European Organization of Research and Treatment of Cancer, Fédération Francophone de Cancérologie Digestive, Gastro-intestinal Tumour Study Group, International Collaborative Cancer Group, Italian Trials in Medical Oncology, Japan Clinical Oncology Group, North Central Cancer Treatment Group, South West Oncology Group, Hospital Clinic Villarroel of Barcelona, Metaxa Cancer Hospital, Jagiellonian of Pireus University, and Medical College of Cracow. We thank Nicolas Thamavong for his help in data management.

The first draft of this manuscript was developed at a meeting of investigators in Sapporo, Japan, September 24–28, 2010. The meeting was supported financially by unrestricted grants from GlaxoSmithKline.

Affiliations of authors: Translational Research and Clinical Trial Center, Hokkaido University Hospital, Sapporo, Japan (KO); Biostatistics dpt, INSEM U900, Institut Curie, Paris, France (XP); Mayo Clinic, Rochester, MN (SA, DS); Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea (Y-JB); SWOG Statistical Center, Seattle, WA (JB); Brussels, Belgium (HB); Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute and Department of Biostatistics, Harvard School of Public Health, Boston, MA (PC); University Clinic Leipzig, University Cancer Center, Leipzig, Germany (FL); Biostatistics and Epidemiology department, Institut Gustave-Roussy, Universite Paris XI, Villejuif, France (SMi, J-PP); Department of Biostatistics, Yokohama City University, Yokohama, Japan (SMo); Department of Biostatistics, School of Public Health, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan (YO); Gastroenterology department, University Hospital Europeen Georges Pompidou, Paris, France (PR); Department of Surgery, Hyogo College of Medicine, Nishinomiya, Japan (MS); Tokai Central Hospital, Kakumuhara, Japan (JS); Department of Gastroenterology and Gastrointestinal Oncology, National Cancer Center Hospital East, Kashiwa, Japan (KS); Digestive Oncology Unit, University Hospital Gasthuisberg, Leuven, Belgium (EVC); International Drug Development Institute, Louvain-la-Neuve, Belgium (MB, TB); I-BioStat, Hasselt University, Diepenbeek, Belgium (MB, TB).

DOI:10.1093/jnci/djt337 JNCI Journal of the National Cancer Institute Advance Access published October 9, 2013

BRIEF COMMUNICATION

Progression-Free Survival as a Surrogate for Overall Survival in Advanced/Recurrent Gastric Cancer Trials: A Meta-Analysis

Xavier Paoletti, Koji Oba, Yung-Jue Bang, Harry Bleiberg, Narikazu Boku, Olivier Bouché, Paul Catalano, Nozomu Fuse, Stefan Michiels, Markus Moehler, Satoshi Morita, Yasuo Ohashi, Atsushi Ohtsu, Arnaud Roth, Philippe Rougier, Junichi Sakamoto, Daniel Sargent, Mitsuru Sasako, Kohei Shitara, Peter Thuss-Patience, Eric Van Cutsem, Tomasz Burzykowski, Marc Buyse; on behalf of the GASTRIC group

Manuscript received February 10, 2013; revised July 25, 2013; accepted July 25, 2013.

Correspondence to: Xavier Paoletti, PhD, Biostatistics Dept / INSERM U900, Institut Curie, 26 rue d'Ulm, 75005 Paris, France (e-mail: xavier.paoletti@curie.net).

The traditional endpoint for assessing efficacy of chemotherapies for advanced/recurrent gastric cancer is overall survival (OS), but OS requires prolonged follow-up. We investigated whether progression-free survival (PFS) is a valid surrogate for OS. Using individual patient data from the GASTRIC meta-analysis, surrogacy of PFS was assessed through the correlation between the endpoints and through the correlation between the treatment effects on the endpoints. External validation of the prediction based on PFS was also evaluated. Individual data from 4069 patients in 20 randomized trials were analyzed. The rank correlation coefficient between PFS and OS was 0.853 (95% confidence interval [CI] = 0.852 to 0.854). The R^2 between treatment effects on PFS and on OS was 0.61 (95% CI = 0.04 to 1.00). Treatment effects on PFS and on OS were only moderately correlated, and we could not confirm the validity of PFS as a surrogate endpoint for OS in advanced/recurrent gastric cancer.

J Natl Cancer Inst

The prognosis of patients with advanced or recurrent gastric cancer (AGC) remains poor, with a 1-year median overall survival (OS) for commonly used chemotherapy regimens, consisting of fluoropyrimidine, platinum, taxane or anthracyclines agents (1). The most important issue in the development of agents for AGC is their ability to prolong OS with acceptable toxicity. Even though median postprogression survival ranges from 5 to 10 months, a validated shorter-term surrogate endpoint would likely reduce drug development costs, sample sizes, or the duration of trials aimed at establishing the benefit of new drugs. Progression-free survival (PFS) is commonly used in phase II and phase III trials. It has been evaluated as a surrogate endpoint for OS in several types of cancers (2–4). The ability to predict clinical benefits on OS from earlier benefits on PFS could be useful at all stages of clinical development. Here, we investigate the surrogacy of PFS for OS within the framework of the GASTRIC meta-analysis (5).

Trials were eligible if they were randomized, closed to accrual before the end of 2006, and collected individual patient data on PFS. To explore the correlation between the treatment effects at the trial level, we relied on the comparison between the experimental arms of the trials included in the meta-analysis with their corresponding control arms. We defined as experimental the treatment that contained the larger number of drugs (eg, triple combinations vs double combinations). In case of equal number of drugs, we defined as experimental the treatment that included the newer agent. When two experimental arms were tested in the same trial, we combined their data for the purposes of the analyses. All data were centrally checked for inconsistencies (6).

We used a meta-analytic validation approach (3,4,7). OS was defined as the time from randomization to death from any cause or to the last follow-up. PFS was the time to tumor progression or death from any cause or time to the last follow-up assessment. A detailed description of statistical methods used is provided in the Supplementary Material (available online). For external validation, we applied the identified relation to predict the hazard ratio (HR) for OS (HR_{OS}) from the hazard ratio for PFS (HR_{PFS}) in randomized trials published since 2000 for which we had not obtained the individual patient data. We extracted the summary statistics for both endpoints (8) and compared the predicted value of HR_{OS} to the one reported in the articles. To determine whether surrogacy also applied to other classes of agents, we extended the validation to three published trials of targeted agents (9–11).

Individual data were obtained on 4069 patients from 20 eligible randomized trials (12–30). The characteristics of the trials have been described elsewhere (5). Thirteen trials defined the progression using radiological criteria, whereas seven used both clinical and radiological assessments. Overall and at the trial level, the treatment effect on PFS (HR = 0.79; 95% confidence interval [CI] = 0.74 to 0.85) tended to be larger than on OS (HR = 0.85; 95% CI = 0.79 to 0.92) as shown on the forest plot of Supplementary Figures 1 and 2 (available online).

The individual-level association, as measured by the rank correlation coefficient, was 0.853 (95% CI = 0.852 to 0.854), indicating substantial correlation between PFS and OS for a given patient. The association at the trial level between log HR_{OS} and log HR_{PFS} was only moderate, with a coefficient of determination, R^2, adjusted for the estimation errors (31), of 0.61 (95% CI = 0.04 to 1.00). The large confidence interval reflects the uncertainty around this estimate. The linear regression model that relates the treatment effect on PFS and on OS adjusted for estimations errors was

$$\log(HR_{OS}) = 0.042 + 0.779 \times \log(HR_{PFS})$$

where the standard errors of the intercept and the slope were 0.79 and 0.295, respectively. This is shown as a straight line in Figure 1. The 95% prediction limits indicate the range of effect on OS that can be expected for a given effect on PFS. The moderate predictive accuracy at the trial level is reflected by the large interval width and a surrogate threshold effect of 0.56; hence, one should observe an HR_{PFS} less than 0.56 to predict, with 95% probability, an HR_{OS} less than 1.

Validation on independent literature data (9–11,32–39) is shown in Table 1 and Supplementary Figure 3 (available online). The larger the number of progressions, the more precise the prediction; however, precision is limited by the variability of the regression line. The observed HR_{OS} fell within the prediction interval in all trials, even in trials using humanized monoclonal antibodies [Trastuzumab (10), bevaxizumab (9), matuzumab (11)]. However, in the trial that concluded a statistically significant benefit of trastuzumab on OS (10), the effect on PFS was smaller than the surrogate threshold effect and therefore could not have been used to predict a statistically significant effect on OS.

This is the first study based on individual patient data to evaluate whether PFS is a reasonable surrogate endpoint to use for randomized trials in AGC. Our results show a high correlation of PFS and OS in individual patients but only a modest correlation ($R^2 = 0.61$) between treatment effects on PFS and OS. It is lower than that found in trials of 5-fluorouracil–based therapies for advanced colorectal cancer (4). The correlation was also lower than in the adjuvant setting (40).

Possible limitations that may explain the moderate correlation observed in our analysis include the numerous processes involved in the progression of stomach cancer (eg, local or distant metastasis, peritoneum involvement), the use of clinical and radiological assessments for progression, and the impact of our definition of investigational treatment related to the heterogeneity in chemotherapies considered here; variability in the investigated treatments and in the effects of the treatments is a condition to generalize any results to future trials. Last, patients included in more recent trials received second-line treatments, including crossover (30), which may have diluted the effect of first-line treatment on OS (2). Because not all trials reported the same information at baseline, we could not assess the surrogacy in clinically relevant subset analyses.

All in all, we would not conclude that PFS is an adequate surrogate for OS in AGC. No precise prediction of the effect of a treatment on OS can be reliably drawn from the effect estimated on PFS.

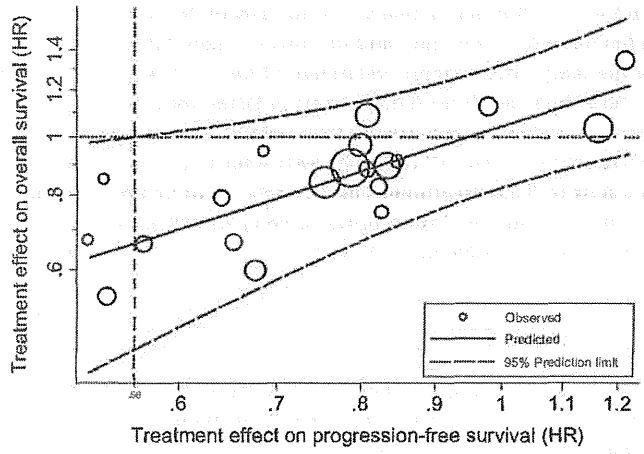


Figure 1. Trial-level association between treatment effects. Log scale was used for the x and y axes; the horizontal line (circles) corresponds to the hazard ratio (HR) on overall survival of 1, which indicates the absence of effect on the overall survival. At the crossing point, the vertical line corresponds to the minimum amount of effect on PFS that will predict a hazard ratio on OS below 1 with 95% probability. This indicates the surrogate threshold effect.

Table 1. Observed and predicted treatment effect on overall survival, based on the observed treatment effect on progression-free survival*

Trial label	Trial	Observed HR_{PFS} (95% CI)	Observed HR_{OS} (95% CI)	Predicted HR_{OS} (95% CI)
A	Jeung et al. (36)	0.63 (0.38 to 1.05)	0.56 (0.35 to 0.88)	0.73 (0.46 to 1.04)
B	AIO (33)	0.67 (0.43 to 1.04)	0.82 (0.47,1.45)	0.76 (0.53 to 1.07)
C	ToGA (10)	0.71 (0.59 to 0.85)	0.74 (0.60 to 0.91)	0.80 (0.58 to 1.09)
D	AVAGAST (9)	0.80 (0.68 to 0.93)	0.87 (0.73 to 1.03)	0.88 (0.76 to 1.14)
E	Kang et al. (35)	0.80 (0.63 to 1.03)	0.85 (0.64 to 1.13)	0.88 (0.76 to 1.14)
F	Park et al. (38)	0.86 (0.54 to 1.37)	0.96 (0.60 to 1.52)	0.93 (0.71 to 1.18)
G	REAL (a)† (34)	0.92 (0.80 to 1.04)	0.92 (0.80 to 1.10)	0.98 (0.77 to 1.22)
H	REAL (b) (34)	0.92 (0.81 to 1.05)	0.86 (0.80 to 0.99)	0.98 (0.77 to 1.22)
I	Ross et al. (39)	0.95 (0.80 to 1.08)	0.91 (0.76 to 1.04)	1.00 (0.79 to 1.29)
J	FLAGS (32)	0.99 (0.86 to 1.14)	0.92 (0.80 to 1.05)	1.03 (0.81 to 1.31)
K	Rao et al. (11)	1.13 (0.63 to 2.01)	1.02 (0.61 to 1.70)	1.14 (0.89 to 1.46)
L	Moehler et al. (37)	1.14 (0.59 to 2.21)	0.77 (0.51 to 1.17)	1.15 (0.90 to 1.48)

* HR = hazard ratio; PFS = progression-free survival; CI = confidence interval; OS = overall survival.

† This trial was designed as a factorial 2 × 2 plan to test two comparisons: a platinum comparison (a) and a fluoropyrimidine comparison (b).

References

1. Oba K, Paoletti X, Bang YJ, et al. Role of chemotherapy for advanced/recurrent gastric cancer: an individual-patient-data meta-analysis. *Eur J Cancer*. 2013;49(7):1565–1577.
2. Broglio KR, Berry DA. Detecting an overall survival benefit that is derived from progression-free survival. *J Natl Cancer Inst*. 2009;101(23):1642–1649.
3. Burzykowski T, Buyse M, Piccart-Gebhart MJ, et al. Evaluation of tumor response, disease control, progression-free survival, and time to progression as potential surrogate end points in metastatic breast cancer. *J Clin Oncol*. 2008;26(12):1987–1992.
4. Buyse M, Burzykowski T, Carroll K, et al. Progression-free survival is a surrogate for survival in advanced colorectal cancer. *J Clin Oncol*. 2007;25(33):5218–5224.
5. GASTRIC. Role of chemotherapy for advanced/recurrent gastric cancer: an individual-patient-data meta-analysis. *Eur J Cancer*. 2013; 49(7):1565–1577
6. Stewart LA, Clarke MJ. Practical methodology of meta-analyses (overviews) using updated individual patient data. Cochrane Working Group. *Stat Med*. 1995;14(19):2057–2079.
7. Burzykowski T, Molenberghs G, Buyse M, Geys H. Validation of surrogate endpoints in multiple randomized clinical trials with failure time endpoints. *J R Stat Soc Series C Appl Stat*. 2001; 50(4): 405–422.
8. Parmar MK, Torri V, Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. *Stat Med*. 1998;17(24):2815–2834.
9. Ohtsu A, Shah MA, Van Cutsem E, et al. Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: a randomized, double-blind, placebo-controlled phase III study. *J Clin Oncol*.; 2011; 29(30):3968–3976.
10. Bang YJ, Van Cutsem E, Feyereislova A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet*. 2010;376(9742):687–697.
11. Rao S, Starling N, Cunningham D, et al. Matuzumab plus epirubicin, cisplatin and capecitabine (ECX) compared with epirubicin, cisplatin and capecitabine alone as first-line treatment in patients with advanced oesophago-gastric cancer: a randomised, multicentre open-label phase II study. *Ann Oncol*.; 2010; 21(11):2213–2219.
12. Douglass HO Jr, Lavin PT, Goudsmit A, Klaassen DJ, Paul AR. An Eastern Cooperative Oncology Group evaluation of combinations of methyl-CCNU, mitomycin C, adriamycin, and 5-fluorouracil in advanced measurable gastric cancer (EST 2277). *J Clin Oncol*. 1984;2(12):1372–1381.
13. Cullinan SA, Moertel CG, Fleming TR, et al. A comparison of three chemotherapeutic regimens in the treatment of advanced pancreatic and gastric carcinoma. Fluorouracil vs fluorouracil and doxorubicin vs fluorouracil, doxorubicin, and mitomycin. *JAMA*. 1985;253(14):2061–2067.
14. Wils JA, Klein HO, Wagener DJ, et al. Sequential high-dose methotrexate and fluorouracil combined with doxorubicin—a step ahead in the treatment of advanced gastric cancer: a trial of the European Organization for Research and Treatment of Cancer Gastrointestinal Tract Cooperative Group. *J Clin Oncol*. 1991;9(5):827–831.
15. Kyoto Research Group for Chemotherapy of Gastric Aancer. A randomized, comparative study of combination chemotherapies in advanced gastric cancer: 5-fluorouracil and cisplatin (FP) versus 5-fluorouracil, cisplatin, and 4'-epirubicin (FPEPIR). *Anticancer Res*. 1992;12(6B):1983–1988.
16. Cullinan SA, Moertel CG, Wieand HS, et al. Controlled evaluation of three drug combination regimens versus fluorouracil alone for the therapy of advanced gastric cancer. North Central Cancer Treatment Group. *J Clin Oncol*. 1994;12(2):412–416.
17. Glimelius B, Ekstrom K, Hoffman K, et al. Randomized comparison between chemotherapy plus best supportive care with best supportive care in advanced gastric cancer. *Ann Oncol*. 1997;8(2):163–168.
18. Vanhoefer U, Rougier P, Wilke H, et al. Final results of a randomized phase III trial of sequential high-dose methotrexate, fluorouracil, and doxorubicin versus etoposide, leucovorin, and fluorouracil versus infusional fluorouracil and cisplatin in advanced gastric cancer: a trial of the European Organization for Research and Treatment of Cancer Gastrointestinal Tract Cancer Cooperative Group. *J Clin Oncol*. 2000;18(14):2648–2657.
19. Kim TW. A prospective randomized phase III trial of 5-fluorouracil and cusplatin (FP) versus epirubicin, cisplatin, and 5-FU (ECF) in the treatment of patients with previously untreated advanced gastric cancer (AGC). *Eur J Cancer*. 2001; 47(3)S314.
20. Ohtsu A, Shimada Y, Shirao K, et al. Randomized phase III trial of fluorouracil alone versus fluorouracil plus cisplatin versus uracil and tegafur plus mitomycin in patients with unresectable, advanced gastric cancer: the Japan Clinical Oncology Group Study (JCOG9205). *J Clin Oncol*. 2003;21(1):54–59.
21. Koizumi W, Fukuyama Y, Fukuda T, et al. Randomized phase II study comparing mitomycin, cisplatin plus doxifluridine with cisplatin plus doxifluridine in advanced unresectable gastric cancer. *Anticancer Res*. 2004;24(4):2465–2470.
22. Pozzo C, Barone C, Szanto J, et al. Irinotecan in combination with 5-fluorouracil and folinic acid or with cisplatin in patients with advanced gastric or esophageal-gastric junction adenocarcinoma: results of a randomized phase II study. *Ann Oncol*. 2004;15(12):1773–1781.
23. Bouche O, Ychou M, Burtin P, et al. Adjuvant chemotherapy with 5-fluorouracil and cisplatin compared with surgery alone for gastric cancer: 7-year results of the FFCD randomized phase III trial (8801). *Ann Oncol*. 2005;16(9):1488–1497.
24. Ajani JA, Fodor MB, Tjulandin SA, et al. Phase II multi-institutional randomized trial of docetaxel plus cisplatin with or without fluorouracil in patients with untreated, advanced gastric, or gastroesophageal adenocarcinoma. *J Clin Oncol*. 2005;23(24):5660–5667.
25. Moehler M, Eimermacher A, Siebler J, et al. Randomised phase II evaluation of irinotecan plus high-dose 5-fluorouracil and leucovorin (ILF) vs 5-fluorouracil, leucovorin, and etoposide (ELF) in untreated metastatic gastric cancer. *Br J Cancer*. 2005;92(12):2122–2128.
26. Thuss-Patience PC, Kretzschmar A, Repp M, et al. Docetaxel and continuous-infusion fluorouracil versus epirubicin, cisplatin, and fluorouracil for advanced gastric adenocarcinoma: a randomized phase II study. *J Clin Oncol*. 2005;23(3):494–501.
27. Van Cutsem E, Moiseyenko VM, Tjulandin S, et al. Phase III study of docetaxel and cisplatin plus fluorouracil compared with cisplatin and fluorouracil as first-line therapy for advanced gastric cancer: a report of the V325 Study Group. *J Clin Oncol*. 2006;24(31):4991–4997.
28. Roth AD, Fazio N, Stupp R, et al. Docetaxel, cisplatin, and fluorouracil; docetaxel and cisplatin; and epirubicin, cisplatin, and fluorouracil as systemic treatment for advanced gastric carcinoma: a randomized phase II trial of the Swiss Group for Clinical Cancer Research. *J Clin Oncol*. 2007;25(22):3217–3223.
29. Dank M, Zaluski J, Barone C, et al. Randomized phase III study comparing irinotecan combined with 5-fluorouracil and folinic acid to cisplatin combined with 5-fluorouracil in chemotherapy naive patients with advanced adenocarcinoma of the stomach or esophagogastric junction. *Ann Oncol*. 2008;19(8):1450–1457.
30. Boku N, Yamamoto S, Fukuda H, et al. Fluorouracil versus combination of irinotecan plus cisplatin versus S-1 in metastatic gastric cancer: a randomised phase 3 study. *Lancet Oncol*. 2009;10(11):1063–1069.
31. Burzykowski T, Molenberghs G, Buyse M. *The Evaluation of Surrogate Endpoints*. New York: Springer; 2006.
32. Ajani JA, Rodriguez W, Bodoky G, et al. Multicenter phase III comparison of cisplatin/S-1 with cisplatin/infusional fluorouracil in advanced gastric or gastroesophageal adenocarcinoma study: the FLAGS trial. *J Clin Oncol*. 2010;28(9):1547–1553.
33. Al-Batran SE, Hartmann JT, Probst S, et al. Phase III trial in metastatic gastroesophageal adenocarcinoma with fluorouracil, leucovorin plus either oxaliplatin or cisplatin: a study of the Arbeitsgemeinschaft Internistische Onkologie. *J Clin Oncol*. 2008;26(9):1435–1442.
34. Cunningham D, Starling N, Rao S, et al. Capecitabine and oxaliplatin for advanced esophagogastric cancer. *N Engl J Med*. 2008;358(1):36–46.
35. Kang YK, Kang WK, Shin DB, et al. Capecitabine/cisplatin versus 5-fluorouracil/

cisplatin as first-line therapy in patients with advanced gastric cancer: a randomised phase III noninferiority trial. *Ann Oncol.* 2009;20(4):666–673.
36. Jeung HC, Rha SY, Im CK, et al. A randomized phase 2 study of docetaxel and S-1 versus docetaxel and cisplatin in advanced gastric cancer with an evaluation of SPARC expression for personalized therapy. *Cancer.* 2011;117(10):2050–2057.
37. Moehler MH, Siebler J, Hoehler T, Janssen J, Wein A, Menges M. CPT11/FA/5-FU versus ELF in chemonaive patients with advanced or metastatic adenocarcinoma of the stomach or gastroesophageal junction: a randomized phase II study. *J Clin Oncol.* 2004; 22(14S): 4064.
38. Park SH, Nam E, Park J, et al. Randomized phase II study of irinotecan, leucovorin and 5-fluorouracil (ILF) versus cisplatin plus ILF (PILF) combination chemotherapy for advanced gastric cancer. *Ann Oncol.* 2008;19(4):729–733.
39. Ross P, Nicolson M, Cunningham D, et al. Prospective randomized trial comparing mitomycin, cisplatin, and protracted venous-infusion fluorouracil (PVI 5-FU) with epirubicin, cisplatin, and PVI 5-FU in advanced esophagogastric cancer. *J Clin Oncol.* 2002;20(8):1996–2004.
40. Burzykowski T, Bang Y. Disease-free survival as a surrogate endpoint for overall survival in an adjuvant trial of curatively resected stomach cancer using individual patient data meta-analysis. *J Clin Oncol.* 2009;27(15s):abstract 4517.

Funding

This work was partially supported by the French Institut National du Cancer (grant PHRC GASTRIC); the Clinical Research Support Unit; and the Epidemiological and Clinical Research Information Network. A meeting was supported financially by unrestricted grants from GlaxoSmithKline. The funders were not present at the meeting, were not involved in the analyses of the data, and did not comment on the present paper.

Notes

X. Paoletti and K. Oba as well as T. Burzykovski and M. Buyse contributed equally to this work T. Burzykowski and M. Buyse contributed equally to this work. A first draft of this manuscript was developed at a meeting of investigators in Sapporo, Japan, September 24–28, 2010. The meeting was supported financially by unrestricted grants from GlaxoSmithKline.

The GASTRIC Investigators: Secretariat: Marc Buyse, Stefan Michiels, Kenichi Nakamura, Koji Oba, Xavier Paoletti, Philippe Rougier, and Seiichiro Yamamoto. Steering Committee: Yung-Jue Bang (Seoul National University College of Medicine, Seoul, Korea); Harry Bleiberg (Jules Bordet Hospital, Brussels, Belgium); Tomasz Burzykowski (Hasselt University, Diepenbeek, Belgium); Marc Buyse (International Drug Development Institute, Louvain-la-Neuve, Belgium);Catherine Delbaldo (Hôpital Louis Mourier, Colombes, France); Stefan Michiels (Institut Gustave Roussy, Université Paris XI, Villejuif, France); Satoshi Morita (Yokohama City University, Kanagawa, Japan); Koji Oba (Hokkaido University Hospital, Hokkaido, Japan); Yasuo Ohashi (University of Tokyo, Tokyo, Japan); Xavier Paoletti (Institut Curie, Paris, France); Jean-Pierre Pignon (Institut Gustave-Roussy, Villejuif, France); Philippe Rougier (University Hospital Europeen Georges Pompidou, Paris, France); Junichi Sakamoto (Tokai Central Hospital, Sohara, Japan); Daniel Sargent (Mayo Clinic, Rochester, MN); Mitsuru Sasako (Hyogo College of Medicine, Hyogo, Japan); and Eric Van Cutsem (Digestive Oncology Unit, University Hospital Gasthuisberf, Leuven, Belgium).Collaborators: J. Ajani, N. Boku, O. Bouche, J. Buckner, C. Coombes, S. Cullinan, M. Dank, N. Fuse, B. Glimelius, R. Hawkins, W. Koizumi, M. Moehler, Y. Nio, A. Ohtsu, A. Roth, K. Shitara, P. Thuss-Patience, A. Tsuburaya, E. Van Cutsem, U. Vanhoefer, J. Wils, and Y. Yamamura. *Writing committee:* Xavier Paoletti, Koji Oba, Tomasz Burzykowski, Yung-Jue Bang, Harry Bleiberg, Narikazu Boku, Olivier Bouché, Paul Catalano, Nozomu Fuse, Stefan Michiels, Markus Moehler, Satoshi Morita, Yasuo Ohashi, Atsushi Ohtsu, Arnaud Roth, Philippe Rougier, Junichi Sakamoto, Daniel Sargent, Mitsuru Sasako, Kohei Shitara, Peter Thuss-Patience, Eric van Cutsem, and Marc Buyse.

This project was initiated under the auspice of the French Institut National du Cancer, who served as a sponsor. The French Institut National du Cancer did not participate in the design of the study. It participated in the conduct of the study at an earlier stage by centralizing all the databases and by providing administrative and data management support. The sponsor had no role in the preparation, review, or approval of the manuscript.

The GASTRIC Group thanks all patients who took part in the trials and contributed to this research. The meta-analysis would not have been possible without their participation or without active participation of the collaborating institutions that provided their trial data (East Central Oncology Group; European Organization of Research and Treatment of Cancer; Fédération Francophone de Cancérologie Digestive; Italian Trials in Medical Oncology; Japanese Cooperative Oncology Group; North Central Cancer Treatment Group; South West Oncology Group; the V325 Study Group; the Swiss Group for Clinical Cancer Research; the Kyoto Research Group for Chemotherapy of Gastric Cancer, the Medizinische Klinik mit Schwerpunkt Haëmatologie und Onkologie, Charité, Universitatsmedizin Berlin; the Center for Caring Sciences, University of Uppsala Sweden. We thank Nicolas Thammavong for the data management.

Affiliations of authors: Biostatistics Department, INSEM U900 Institut Curie, Paris, France (XP); Translational Research and Clinical Trial Center, Hokkaido University Hospital, Hokkaido, Japan (KO); Seoul National University College of Medicine, Oncology Division, Seoul, Korea (YJB); Jules Bordet Hospital, Brussels, Belgium (HB); St. Marianna University School of Medicine, Kawasaki, Japan (NB); Hôpital Robert Debré, Reims, Department of Clinical Oncology, France (OB); Dana-Farber Cancer Institute and Harvard School of Public Health, Department of Biostatistics, Boston, MA (PC); National Cancer Center Hospital East, Department of Gastrointestinal Oncology, Kashiwa, Japan (NF, AO); Institut Gustave Roussy, Université Paris XI, Biostatistics and Epidemiology Department, Villejuif, France (SMi); Johannes Gutenberg University, Medical Department, Mainz, Germany (MM); Yokohama City University, Department of Biostatistics and Epidemiology, Kanagawa, Japan (SMo); University of Tokyo, Tokyo, Japan (YO); University Hospital, Department of Surgery, Geneva, Switzerland (AR); University Hospital Europeen Georges Pompidou, Gastro-enterology Department, Paris, France (PR); Tokai Central Hospital, Sohara, Japan (JS); Mayo Clinic, Division of Biomedical Statistics and Informatics, Rochester, MN (DS); National Cancer Center Hospital East, Kashiwa, Japan (MS); Aichi Cancer Center Hospital, Department of Gastrointestinal Oncology, Aichi, Japan (KS); Charité-Universitätsmedizin Berlin, Department of Haematology, Oncology, and Tumorimmunology, Berlin, Germany (PT-P); University Hospital Gasthuisberg, Digestive Oncology Unit, Leuven, Belgium (EVC); Hasselt University, Interuniversity Institute for Biostatistics and Statistical Bioinformatics, Diepenbeek, Belgium (TB, MB); International Drug Development Institute, Louvain-la-Neuve, Belgium (MB).

Cancer Science
The official journal of the Japanese Cancer Association JCA

*UGT1A1*6, 1A7*3,* and *1A9*22* genotypes predict severe neutropenia in FOLFIRI-treated metastatic colorectal cancer in two prospective studies in Japan

Shoichi Hazama,[1,15] Hideyuki Mishima,[2] Ryouichi Tsunedomi,[1] Yusuke Okuyama,[3] Takeshi Kato,[4] Ken-ichi Takahashi,[5] Hiroshi Nozawa,[6] Hideaki Ando,[7] Michiya Kobayashi,[8] Hiroyoshi Takemoto,[9] Naoki Nagata,[10] Shinsuke Kanekiyo,[1] Yuka Inoue,[1] Yoshihiko Hamamoto,[11] Yusuke Fujita,[11] Yuji Hinoda,[12] Naoko Okayama,[12] Koji Oba,[13] Jun-ichi Sakamoto[14] and Masaaki Oka[1]

[1]Department of Digestive Surgery and Surgical Oncology (Surgery II), Yamaguchi University Graduate School of Medicine, Ube; [2]Cancer Center, Aichi Medical University, Nagakute; [3]Department of Digestive Medicine, Kyoto First Red Cross Hospital, Kyoto; [4]Department of Surgery, Kansai Rosai Hospital, Osaka; [5]Department of Surgery, Aomori Prefectural Hospital, Aomori; [6]Nozawa Clinic, Imizu; [7]Program for the Strategic Education of Future Surgeons, Akita University Graduate School of Medicine, Akita; [8]Cancer Treatment Center, Kochi Medical School, Kochi; [9]Department of Surgery, Kinki Central Hospital, Osaka; [10]Department of Surgery, Kitakyushu General Hospital, Kokura; [11]Biomolecular Engineering of Applied Molecular Bioscience; [12]Oncology and Laboratory Medicine, Yamaguchi University Graduate School of Medicine, Ube; [13]Translational Research and Clinical Trial Center, Hokkaido University Hospital, Sapporo; [14]Tokai Central Hospital, Gifu, Japan

(Received July 21, 2013/Revised September 2, 2013/Accepted September 5, 2013/Accepted manuscript online September 13, 2013/Article first published online October 27, 2013)

Retrospective studies have suggested that UDP-glucuronosyltransferase *(UGT)1A1*, *UGT1A7*, and *UGT1A9* predict severe toxicity and efficacy of irinotecan-containing regimens. We prospectively evaluated the impact of *UGT1A* genotypes and haplotypes on severe toxicity and efficacy in patients treated with fluorouracil, leucovorin, and irinotecan combination chemotherapy (FOLFIRI) for metastatic colorectal cancer (mCRC) from the two prospective multicenter phase II studies in Japan. The FLIGHT1 study was a first-line FOLFIRI trial, and FLIGHT2 was a FOLFOX-refractory, second-line FOLFIRI trial. A total of 73 patients agreed to additional analysis, and were genotyped for *UGT1A* polymorphisms, *UGT1A1*28* (TA6>TA7), *UGT1A1*6* (211G>A), *UGT1A1*27* (686C>A), *UGT1A1*60* (−3279T>G), *UGT1A1*93* (−3156G>A), *UGT1A7* (−57T>G), *UGT1A7*3* (387T>G, 622T>C), and *UGT1A9*22* (T9>T10). Of 73 patients, 34 developed G3/4 severe hematological toxicities. The toxicities were significantly more frequent in patients with *UGT1A1*6* (211A), *UGT1A7* (387G), and *UGT1A9*22* reference alleles (T9). Haplotype I, which consists of all favorable alleles, was associated with a significant reduction in hematologic toxicity ($P = 0.031$). In contrast, haplotype II, which contains four high-risk alleles, showed significantly higher hematologic toxicity than the other haplotypes ($P = 0.010$). Six out of seven patients who were homozygous for *UGT1A1*28* or **6* experienced severe hematological toxicity despite the fact that their response rate was not impaired (42.9%). We concluded that *UGT1A* polymorphisms, especially *UGT1A1*6*, are important for the prediction of severe toxicity of FOLFIRI in northeast Asian populations. In this regard, haplotype analyses should substantially impact the prediction of severe hematological toxicities of FOLFIRI. (Clinical Trial Registration: UMIN000002388 and UMIN000002476). (*Cancer Sci* 2013; 104: 1662–1669)

Irinotecan with continuous fluorouracil plus leucovorin (FOLFIRI) has been approved as a first-line therapy for metastatic colorectal cancer (mCRC).[1–3] Although this regimen can result in prolonged survival, 20–35% of FOLFIRI-treated patients develop severe neutropenia. Irinotecan is activated by hydrolysis to SN-38, a potent topoisomerase I inhibitor that is primarily inactivated through biotransformation into SN-38 glucuronide (SN-38G) by UDP-glucuronosyltransferase (UGT) 1A1.[4,5] The toxicity of irinotecan is correlated to polymorphisms in the number of TA repeats in the promoter region of *UGT1A1*28* that affect transcriptional efficiency.[6] Other *UGT1A* polymorphisms are related to the efficiency of the detoxification of SN-38. The *UGT1A9* isoform contributes to SN-38 glucuronidation.[7] The **22* variant (a T insert at position_118) of hepatic *UGT1A9* is associated with increased gene expression[8] and reduced hematologic toxicity.[9] The *UGT1A7* isoform is mainly expressed in the gastrointestinal tract, and a gene variant with impaired enzyme function is *UGT1A7*3*. The effect of the *UGT1A7* polymorphisms on irinotecan pharmacology has been studied.[10–13] Recently, the toxicity and tumor response of FOLFIRI has been correlated with the *UGT1A1*, *UGT1A7*, and *UGT1A9* genetic variants and their haplotypes in Caucasians patients.[9,14] There are ethnic differences in *UGT1A* genes and their linkage disequilibrium (LD) between Caucasian and Asian populations. *UGT1A1*6* and *UGT1A1*27* were strongly associated with severe neutropenia, especially among Asian patients.[11,12,15,16] The relative contribution of polymorphisms, especially *UGT1A1*6*, to the prediction of the outcome of FOLFIRI therapy in Asian patients must be determined. Moreover, the location of these variants in the same *UGT1A* gene cluster and their LD indicate that haplotype-based studies should be carried out to determine the potential interaction among *UGT1A* variants. Hence, the aim of this study is to evaluate whether *UGT1A* alleles might be involved in the risk of toxicity and response in Japanese mCRC patients treated with FOLFIRI.

Patients and Methods

Study design and patient eligibility. The objective of this study was to assess the relationship between *UGT1A* polymorphisms and toxicity as well as tumor response. The study was carried out as an ancillary investigation of two prospective studies that involved 20 treatment centers in Japan. The study participants have been described in detail elsewhere.[17,18] This study was carried out according to the Declaration of Helsinki. The study protocol was approved by the Ethics and Scientific Committee and the institutional review board of each participating institution, and all patients gave written informed consent before entering the study. Eligibility criteria included:

[15]To whom correspondence should be addressed.
E-mail: hazama@yamaguchi-u.ac.jp

doi: 10.1111/cas.12283

histologically proven mCRC; no prior chemotherapy for metastatic disease in the case of FLIGHT1[17] (UMIN000002388), or resistance to FOLFOX as the first-line chemotherapy in the case of FLIGHT2[18] (UMIN000002476); aged 20–80 years; Eastern Cooperative Oncology Group performance status of 0–1; at least one measurable lesion according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0[19]; and adequate organ function.

Thirty-nine of 53 patients from the FLIGHT1 study and 36 of 50 patients from the FLIGHT2 study agreed to the additional investigation. Two patients who were homozygous for *UGT1A1*28* and received a reduced dosage of irinotecan were excluded from the whole analysis of toxicities and efficacies and analyzed individually.

Treatment. All patients were treated with the modified FOLFIRI regimen as described by Tournigand *et al.*[3] Briefly, the regimen consisted of irinotecan 150 mg/m^2 (instead of 180 mg/m^2 commonly used in North America and Europe) given i.v. for 90 min on day 1 + 400 mg/m^2 fluorouracil bolus followed by 2400 mg/m^2 fluorouracil continuous infusion during 46 h + 200 mg/m^2 leucovorin on day 1 every 2 weeks. Blood tests and clinical evaluation were carried out every 2 weeks before treatment. Chemotherapy could be given if patients had a leukocyte count >3000 mm^3, a neutrophil count >1500 mm^3, a platelet count >75 000 mm^3, and clinical toxicity that was resolved or grade 1. In patients who were homozygous for *UGT1A1*28*, the starting dose of irinotecan was reduced to 100 mg/m^2, based on the recommendation of an advisory meeting by the subcommittee of the Food and Drug Administration Center for Drug Evaluation and Research held November 2004[20] and our previous phase I study.[21] Two patients were homozygous for **28* and therefore excluded from the whole analysis of toxicities and efficacies and analyzed individually.

Efficacy and toxicity assessment. Objective clinical evaluation, blood counts, and hepatic and renal function tests were carried out within 48 h before each cycle. Computed tomography scans of measurable lesions were assessed at baseline and then repeated at least every four cycles. Objective tumor response and duration of response were assessed by RECIST version 1.0. Toxicity was evaluated according to Common Toxicity Criteria for Adverse Events version 3.0.

Uridine diphosphate–glucuronosyltransferase genetic analyses. Genomic DNA was extracted from peripheral blood anticoagulated with EDTA-2Na using a conventional NaI method.[22] *UGT1A1*28* (TA6>TA7), UGT1A7*3 (387T>G, 622T>C)/*UGT1A9*22* (**1b* [T9>T10]), and *UGT1A1*93* (−3156G>A)/*UGT1A1*6* (211G>A)/*UGT1A1*27* (686C>A) /*UGT1A1*60* (−3279T>G)/*UGT1A7* (−57T>G) were genotyped as described previously by fragment size analysis, direct sequencing, and TaqMan assay, respectively.[21,23] *UGT1A7*1*, *UGT1A7*2*, *UGT1A7*3*, and *UGT1A7*4* can be tagged by sequencing of 387T>G and 622T>C.[24] For fragment size analysis, PCR reactions were carried out in a total volume of 10 μL containing template DNA (80 ng/μL), according to the manufacturer's instructions (Ex Taq; Takara, Tokyo, Japan). The amplification was carried out with a GeneAmp PCR System PC808 (ASTEC, Tokyo, Japan) with an initial denaturation at 95°C for 2 min followed by 27 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 20 s, and extension at 72°C for 30 s. The PCR products of TA6 and TA7, whose sizes were 94 and 96 bp, respectively, were mixed with Hi-Di formamide, including the internal size standard (GeneScan 500; Applied Biosystems, Foster City, CA, USA) at a 1:10 ratio. Then, samples were run in the ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Fragment sizes were determined by comparison with the internal size standard (GeneScan LIZ-500) by using the local Southern algorithm, and the data were analyzed by GeneMapper software version 3.5 (Applied Biosystems). For direct sequencing, PCR amplifications were carried out by using the Gene Amp PCR System PC808 (ASTEC) with Ex Taq polymerase. Amplification conditions were 30 cycles of 95°C for 30 s, each annealing temperature for 20 s, and 72°C for 30 s. The PCR products were purified using ExoSAP-IT (Amersham Bioscience, Tokyo, Japan) for 20 min at 37°C and then for 20 min at 80°C. Sequencing reactions were carried out by using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Tokyo, Japan). After purification with ethanol, the reaction products were analyzed by an ABI 3100-Avant Genetic Analyzer (Applied Biosystems). TaqMan assays of PCR products were carried out according to the manufacturer's protocol. Specific forward/reverse PCR primers and TaqMan probes for *UGT1A1*93* were custom synthesized by Applied Biosystems. Primers and probes for *UGT1A1*6*, *UGT1A1*27*, *UGT1A1*60*, and *UGT1A7* (−57) were purchased from Applied Biosystems (TaqMan SNP Genotyping Assays). Reaction mixtures were loaded into 384-well plates and placed in the ABI Prism 7900HT Sequence Detection System (Applied Biosystems). The PCR amplifications were carried out as follows: initial denaturation at 95°C for 10 min, followed by 40 cycles of PCR with a denaturation at 95°C for 15 s, and one-step annealing/extension for 1 min at 60°C.

Statistical analysis. These analyses are an additional investigation of the FLIGHT1[17] and FLIGHT2[18] studies, which were prospectively designed to evaluate the associations between *UGT1A* genotypes/haplotypes and outcomes of toxicity (severe toxicity at first cycle and in all cycles) and efficacy (response rate [complete response, partial response] and progression-free survival [PFS]). In the present study, we evaluated associations between genotypes/haplotypes as well as clinical variables (sex, age, and performance status), toxicity, and response rate by Fisher's exact test and the Cochran–Armitage trend test (C.A.-test). Logistic regression models were investigated for toxicity. Genetic variants were included in a stepwise logistic regression analysis in the multivariate model. Calculations were carried out by using R version 2.13.0 software.[25] Each nucleotide polymorphism was evaluated for Hardy–Weinberg equilibrium and LD, and case–control haplotype analyses were carried out with Haploview 4.2 software.[26] Lewontin's coefficient D' and correlation coefficient r^2 were calculated as measures of LD. $P < 0.05$ was considered statistically significant.

Results

Clinical features, genotype/haplotype frequency, and LD. In the 75 Japanese colorectal cancer patients, genotypes of *UGT1A1*28* and *UGT1A1*93* as well as *UGT1A7* (622T>C) and *UGT1A7* (−57T>G) matched perfectly, so *UGT1A1*93* and *UGT1A7* (−57T>G) were excluded from further examination (Table 1, Fig. 1). Minor allele frequencies of seven remaining *UGT1A* polymorphisms are shown in Table S1. The *P*-values of all seven *UGT1A* polymorphisms were >0.05, under the Hardy–Weinberg equilibrium. High LD was also observed between *UGT1A1*6* and *UGT1A7* (622T>C), *UGT1A1*6* and *UGT1A7* (−57T>G), and *UGT1A7* (387T>G) and *UGT1A9*22(*1b)* (Fig. 1).

The frequency of *UGT1A1*28* was lower (Table 1) in the Japanese patients than Caucasians as reported previously.[9,27] *UGT1A1*6* frequency was discriminatively high, but it was rare among Caucasians.[28] The frequency of *UGT1A1*60* and *UGT1A7*3* was low in Japanese patients, *UGT1A9*22* had the same frequency in Japanese and Caucasian patients, and *UGT1A1*27* had very low frequency and no homozygosity.

Table 1. Clinical features and genotype frequencies in Japanese patients with metastatic colorectal cancer

Clinical feature or genotype	Detail	Frequency (%)
Sex	Male	50 (66.7)
	Female	25 (33.3)
Age, years	≤60	28 (37.3)
	>60	47 (62.7)
Performance status†	0	58 (77.3)
	1	17 (22.7)
Hematologic toxicity (entire course)	No	40 (53.3)
	Yes	35 (46.7)
Hematologic toxicity(first cycle)	No	63 (84.0)
	Yes	12 (16.0)
Objective response	CR + PR	24 (33.3)
	SD + PD	48 (66.7)
*UGT1A1*6*	–/–	50 (66.7)
	–/**6*	23 (30.7)
	**6/*6*	2 (2.7)
*UGT1A1*27*	–/–	73 (97.3)
	–/**27*	2 (2.7)
*UGT1A1*28*	–/–	59 (78.7)
	–/**28*	14 (18.7)
	**28/*28*	2 (2.7)
*UGT1A1*60*	–/–	40 (53.3)
	–/**60*	29 (38.7)
	**60/*60*	6 (8.0)
*UGT1A1*93*	–/–	59 (78.7)
	–/**93*	14 (18.7)
	**93/*93*	2 (2.7)
UGT1A7 (–57T>G)	–57T/T	40 (53.3)
	–57T/G	29 (38.7)
	–57G/G	6 (8.0)
*UGT1A7*3* (387T>G)	387T/T	25 (33.3)
	387T/G	37 (49.3)
	387G/G	13 (17.3)
*UGT1A7*3* (622T>C)	622T/T	40 (53.3)
	622T/C	29 (38.7)
	622C/C	6 (8.0)
*UGT1A9*22* (*UGT1A9*1b*)	**22/*22*	26 (34.7)
	–/**22*	36 (48.0)
	–/–	13 (17.3)

†Assessed using Eastern Cooperative Oncology Group criteria.
CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

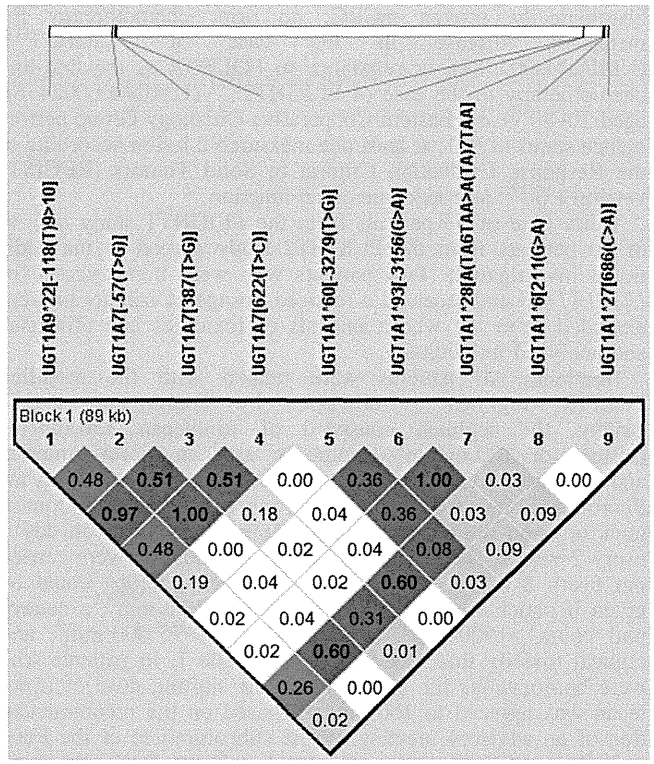


Fig. 1. Pairwise linkage disequilibrium relationships between *UGT1A* polymorphisms in Japanese patients with metastatic colorectal cancer. Lewontin's coefficient D′ is represented by the color scheme: log of the odds (LOD) ≥ 2 shown in pink/red; LOD < 2 and D′ = 1 shown in blue; and LOD < 2 and D′ < 1 shown in white. The correlation coefficient r^2 is shown in each box.

Table 2. *UGT1A* haplotype frequencies in Japanese patients with metastatic colorectal cancer

Hp	*UGT1A1*				*UGT1A7*		*UGT1A9*	
	**60*	**28*	**6*	**27*	387T>G	622T>C	**22*	Frequency
I	T	TA_6	G	C	T	T	T_{10}	0.520
II	T	TA_6	A†	C	G†	C†	T_9†	0.173
III	G†	TA_6	G	C	G†	T	T_9†	0.127
IV	G†	TA_7†	G	C	G†	C†	T_9†	0.053
V	G†	TA_7†	G	C	T	T	T_{10}	0.053
VI	T	TA_6	G	C	G†	C†	T_9†	0.020
VII	G†	TA_7†	G	A†	G†	T	T_9†	0.013
VIII	G†	TA_6	G	C	G†	C†	T_9†	0.013
IX	G†	TA_6	G	C	T	T	T_{10}	0.013

†Association allele was estimated by Haploview. Hp, haplotype.

Haplotype frequencies are listed in Table 2. The major haplotypes in the Japanese population were I, II, and III, and our analyses were performed on these.

***UGT1A* genotypes/haplotypes and severe toxicity.** Correlations between *UGT1A* genotypes/haplotypes and severe (grade 3–4) toxicity were analyzed. Of 73 patients, 34 had G3/4 hematological toxicity. Severe hematological toxicity during the entire course of therapy was more frequent in patients with *UGT1A1*6* (211A, $P = 0.018$ by C.A.-test) and *UGT1A7* (387G, $P = 0.039$ by C.A.-test) alleles than in patients without these variant alleles. In the *UGT1A9*22* polymorphism, the variant T10 allele was a marker for reduced toxicity ($P = 0.028$ by C.A.-test), as previously reported.[8,9] Severe hematologic toxicity was trend toward higher among the *UGT1A7* (622C) allele ($P = 0.058$ by Fisher's exact test). For severe hematological toxicity during the entire course of therapy, multivariate analysis indicated that *UGT1A1*6* was the only significant predictor ($P = 0.022$, odds ratio 3.00, 95% confidence interval 1.17–7.69; Table S5). Older age (60 years or more) was also a risk factor for severe toxicity (Table 3).

Haplotype I, which consisted of all favorable alleles, was associated with low hematologic toxicity both after the first cycle ($P = 0.080$ by C.A.-test) and during the entire course of therapy ($P = 0.031$ by C.A.-test). In contrast, haplotype II, which contains four high-risk alleles, was significantly associated with high hematologic toxicity during the entire course of therapy ($P = 0.010$ by C.A.-test) (Table 3).

In the trial of first-line therapy (FLIGHT1, $n = 38$, Table 4), *UGT1A1*28* ($P = 0.063$ by Fisher's exact test) genotypes were

doi: 10.1111/cas.12283

Table 3. *UGT1A* genotypes/clinical features and severe hematologic toxicity in Japanese patients with metastatic colorectal cancer treated with FOLFIRI

Genotype or clinical feature	Detail	Severe hematologic toxicity							
		After first cycle				During entire course of therapy			
		Toxicity		*P*-value		Toxicity		*P*-value	
		Yes	No	Fisher's exact test	C.A.-trend test	Yes	No	Fisher's exact test	C.A.-trend test
*UGT1A1*6*	–/–	6	42	0.175	0.132	18	30	0.049	0.018
	–/**6*	5	18			14	9		
	**6/*6*	1	1			2	0		
*UGT1A1*27*	–/–	11	60	0.304	ND	33	38	1.000	ND
	–/**27*	1	1			1	1		
*UGT1A1*28*	–/–	8	51	0.227	ND	27	32	1.000	ND
	–/**28*	4	10			7	7		
*UGT1A1*60*	–/–	4	36	0.114	0.313	16	24	0.402	0.279
	–/**60*	8	21			16	13		
	**60/*60*	0	4			2	2		
UGT1A7 (387T>G)	387T/T	2	23	0.360	0.328	7	18	0.079	0.039
	387T/G	8	28			20	16		
	387G/G	2	10			7	5		
UGT1A7 (622T>C)	622T/T	4	36	0.191	0.162	14	26	0.058	0.105
	622T/C	7	21			18	10		
	622C/C	1	4			2	3		
*UGT1A9*22 (UGT1A9*1b)*	**22/*22*	2	24	0.356	0.296	7	19	0.044	0.028
	–/**22*	8	27			20	15		
	–/–	2	10			7	5		
Haplotype I	0	3	12	0.041	0.080	9	6	0.072	0.031
	1	9	29			20	18		
	2	0	20			5	15		
Haplotype II	0	6	43	0.170	0.106	18	31	0.025	0.010
	1	5	17			14	8		
	2	1	1			2	0		
Haplotype III	0	10	45	0.765	0.449	24	31	0.488	0.282
	1	2	15			9	8		
	2	0	1			1	0		
Sex	Male	8	40	1.000	ND	22	26	1.000	ND
	Female	4	21			12	13		
Age, years	≤60	2	26	0.114	ND	8	20	0.018	ND
	>60	10	35			26	19		
PS	0	9	48	0.718	ND	27	30	1.000	ND
	1	3	13			7	9		

–, reference allele. C.A., Cochran–Armitage; ND, not done; PS, Eastern Cooperative Oncology Group performance status.

risk factors for severe hematological toxicity after the first cycle, and the allele number of *UGT1A7* (387G, $P = 0.026$ by C.A.-test) and *UGT1A9*22* (T10, $P = 0.016$ by C.A.-test) were risk factors for severe hematological toxicity during the entire course of therapy.

In the trial of second-line therapy (FLIGHT2, $n = 35$, Table 5), older age (60 years and older, $P = 0.069$ by Fisher's exact test) trends to a risk factor for severe hematological toxicity after the first cycle. The severe hematological toxicity during the entire course of therapy was significantly different among the genotypes of *UGT1A7* (387G, $P = 0.047$ by Fisher's exact test), *UGT1A9*22* (T9, $P = 0.047$ by Fisher's exact test), and patient age ($P = 0.036$ by Fisher's exact test).

Only two patients, one in FLIGHT1 and one in FLIGHT2, developed grade 3 diarrhea. Both of them had UGT1A diplotype I/II.

***UGT1A* genotypes/haplotypes and response/PFS.** Objective tumor responses were analyzed in 71 patients. Of 36 patients, 18 had complete or partial tumor responses in FLIGHT 1, and 6 out of 35 patients had objective responses in FLIGHT 2. No genotype/haplotype was associated with tumor response in FLIGHT 1. In FLIGHT2, the numbers of *UGT1A1*6* and haplotype II were positive factors ($P = 0.021$ by C.A.-test) and *UGT1A1*60* was a negative factor ($P = 0.041$ by C.A.-test) for objective responses (Table 5).

Effects of UGT1A polymorphisms on PFS in FLIGHT1 and FLIGHT2 were analyzed. There was no association between UGT1A polymorphisms and PFS. Only female sex was the negative factor for PFS ($P = 0.016$) (Table S6).

***UGT1A1*28* and *UGT1A1*6* homozygous.** Homozygosity of *UGT1A1*28* or **6* and compound heterozygosity of **28* and **6* were reported as high-risk factors for hematologic toxicity, especially in Asia,[29] therefore, we examined these genotypes (Table S7). Although the initial dose of irinotecan was reduced to 100 mg/m^2 for patients with homozygous *UGT1A1*28*, one of two patients suffered from severe toxicity. In all cases, six of seven patients suffered from severe hematological toxicity. Although in these patients the toxicity was very severe, the response rate was not impaired (3/7, 42.9%).

Discussion

The present study evaluated whether *UGT1A* polymorphisms influence the toxicity and efficacy of the FOLFIRI regimen in

Table 4. Associations between *UGT1A* genotypes and irinotecan toxicity and objective responses in Japanese patients with metastatic colorectal cancer treated with FOLFIRI as first-line therapy (FLIGHT1)

Genotype or clinical feature	Detail	Severe hematologic toxicity								Objective responses			
		After first cycle				During entire course of therapy				During entire course of therapy			
		Toxicity		*P*-value		Toxicity		*P*-value		Responses		*P*-value	
		Yes	No	Fisher's exact test	C.A.- trend test	Yes	No	Fisher's exact test	C.A.- trend test	CR + PR	SD + PD	Fisher's exact test	C.A.- trend test
*UGT1A1*6*	*–/–*	4	21	0.243	0.266	10	15	0.384	0.150	11	12	0.725	1.000
	*–/*6*	2	10			7	5			7	5		
	**6/*6*	1	0			1	0			0	1		
*UGT1A1*27*	*–/–*	6	31	0.184	ND	17	20	0.474	ND	17	18	1.000	ND
	*–/*27*	1	0			1	0			1	0		
*UGT1A1*28*	*–/–*	4	28	0.063	ND	14	18	0.395	ND	16	15	1.000	ND
	*–/*28*	3	3			4	2			2	3		
*UGT1A1*60*	*–/–*	1	18	0.079	0.133	7	12	0.206	0.095	8	11	0.109	0.781
	*–/*60*	6	11			9	8			10	5		
	**60/*60*	0	2			2	0			0	2		
UGT1A7 (387T>G)	387T/T	1	12	0.490	0.213	4	9	0.065	0.026	7	5	0.678	0.815
	387T/G	4	14			8	10			7	10		
	387G/G	2	5			6	1			4	3		
UGT1A7 (622T>C)	622T/T	2	19	0.113	0.079	7	14	0.132	0.102	10	10	1.000	1.000
	622T/C	4	11			10	5			7	7		
	622C/C	1	1			1	1			1	1		
*UGT1A9*22 (UGT1A9*1b)*	**22/*22*	1	13	0.413	0.183	4	10	0.057	0.016	8	5	0.474	0.646
	*–/*22*	4	13			8	9			6	10		
	–/–	2	5			6	1			4	3		
Haplotype I	0	3	5	0.100	0.040	7	1	0.028	0.019	4	4	1.000	1.000
	1	4	16			8	12			9	9		
	2	0	10			3	7			5	5		
Haplotype II	0	4	22	0.249	0.202	10	16	0.212	0.080	12	12	1.000	0.755
	1	2	9			7	4			6	5		
	2	1	0			1	0			0	1		
Haplotype III	0	6	22	0.720	0.398	12	16	0.568	0.253	13	13	1.000	0.747
	1	1	8			5	4			5	4		
	2	0	1			1	0			0	1		
Sex	Male	5	19	1.000	ND	12	12	0.745	ND	13	10	0.489	ND
	Female	2	12			6	8			5	8		
Age, years	≤60	2	12	1.000	ND	5	9	0.328	ND	6	7	1.000	ND
	>60	5	19			13	11			12	11		
PS	0	5	28	0.223	ND	15	18	0.653	ND	17	15	0.603	ND
	1	2	3			3	2			1	3		

–, reference allele. C.A., Cochran–Armitage; CR, complete response; ND, not done; PD, progressive disease; PR, partial response; PS, Eastern Cooperative Oncology Group performance status; SD, stable disease.

doi: 10.1111/cas.12283

Table 5. Associations between *UGT1A* genotypes and irinotecan toxicity and objective responses in Japanese patients with metastatic colorectal cancer treated with FOLFIRI as second-line therapy (FLIGHT2)

Genotype or clinical feature	Detail	Severe hematologic toxicity								Objective responses			
		After first cycle				During entire course of therapy				During entire course of therapy			
		Toxicity		*P*-value		Toxicity		*P*-value		Responses		*P*-value	
		Yes	No	Fisher's exact test	C.A.-trend test	Yes	No	Fisher's exact test	C.A.-trend test	CR + PR	SD + PD	Fisher's exact test	C.A.-trend test
*UGT1A1*6*	–/–	2	21	0.400	0.306	8	15	0.105	0.054	2	21	0.058	0.021
	–/**6*	3	8			7	4			3	8		
	**6/*6*	0	1			1	0			1	0		
*UGT1A1*27*	–/–	5	29	1.000	ND	16	18	1.000	ND	6	28	1.000	ND
	–/**27*	0	1			0	1			0	1		
*UGT1A1*28*	–/–	4	23	1.000	ND	13	14	0.700	ND	6	21	0.299	ND
	–/**28*	1	7			3	5			0	8		
*UGT1A1*60*	–/–	3	18	1.000	0.819	9	12	0.414	0.859	6	15	0.106	0.041
	–/**60*	2	10			7	5			0	12		
	**60/*60*	0	2			0	2			0	2		
UGT1A7 (387T>G)	387T/T	1	11	0.528	1.000	3	9	0.047	0.542	2	10	1.000	0.893
	387T/G	4	14			12	6			3	15		
	387G/G	0	5			1	4			1	4		
UGT1A7 (622T>C)	622T/T	2	17	0.612	0.831	7	12	0.403	0.491	2	17	0.317	0.228
	622T/C	3	10			8	5			3	10		
	622C/C	0	3			1	2			1	2		
*UGT1A9*22 (UGT1A9*1b)*	**22/*22*	1	11	0.528	1.000	3	9	0.047	0.542	2	10	1.000	0.893
	–/**22*	4	14			12	6			3	15		
	–/–	0	5			1	4			1	4		
Haplotype I	0	0	7	0.095	0.765	2	5	0.049	0.501	1	6	1.000	0.753
	1	5	13			12	6			3	15		
	2	0	10			2	8			2	8		
Haplotype II	0	2	21	0.400	0.306	8	15	0.105	0.054	2	21	0.058	0.021
	1	3	8			7	4			3	8		
	2	0	1			1	0			1	0		
Haplotype III	0	4	23	1.000	ND	12	15	1.000	ND	6	21	0.299	ND
	1	1	7			4	4			0	8		
	2	0	0			0	0			0	0		
Sex	Male	3	21	0.640	ND	10	14	0.716	ND	4	20	1.000	ND
	Female	2	9			6	5			2	9		
Age, years	≤60	0	14	0.069	ND	3	11	0.036	ND	2	12	1.000	ND
	>60	5	16			13	8			4	17		
PS	0	4	20	1.000	ND	12	12	0.493	ND	5	19	0.640	ND
	1	1	10			4	7			1	10		

–, reference allele. C.A., Cochran–Armitage; CR, complete response; ND, not done; PD, progressive disease; PR, partial response; PS, Eastern Cooperative Oncology Group performance status; SD, stable disease.

Japanese mCRC patients. This study was carried out as an integrated investigation of two prospective studies that involved 20 treatment centers in Japan.[17,18] To the best of our knowledge, this is the first prospective study to assess the role of *UGT1A* polymorphisms on the efficacy and toxicity of FOLFIRI.

The *UGT1A1*, *UGT1A7*, and *UGT1A9* genetic variants and their haplotypes play a predictive role in the outcome of FOLFIRI treatments in Caucasian mCRC patients.[9] There are ethnic differences in *UGT1A* genotypes. Among the Japanese population, *UGT1A1*28* has a low frequency and *UGT1A1*6* has a discriminatively high frequency, unlike the frequencies among Caucasians.[11,12,16,17] Therefore, the relationship between *UGT1A* genetics and the outcome of FOLFIRI should be evaluated in each ethnic group.

In our analysis of Japanese colorectal cancer patients treated with FOLFIRI, severe hematological toxicity during the entire course of therapy was more frequent in patients with *UGT1A1*6* (211A) and *UGT1A7*3* (387G) genotypes. In contrast, the variant T10 allele of the *UGT1A9*22* (*1b) polymorphism was a marker for reduced toxicity (Table 3).[8,9] In addition to certain genotypes, older age was also a risk factor for severe hematological toxicity. Unlike findings from Caucasian patients, the *UGT1A1*28* allele was not necessarily related to hematological toxicity in Japanese patients. On the contrary, *UGT1A1*6* was strongly correlated with severe hematological toxicity, which is consistent with other clinical studies of Asian populations.[11,12,16] We can assume that both *UGT1A9*22* (reference T9 genotype) and *UGT1A7*3* (387G) are risk alleles that have strong LD (correlation coefficient $r^2 = 0.97$) for each other (Fig. 1), as previously reported.[9] Interestingly, there was no significant relationship between hematological toxicity and female sex, unlike the previous findings of Cecchin *et al.*[9] for Caucasians.

The close LD between *UGT1A1*6* and *UGT1A7*3* (387T>G, 0.32; 622T>C, 0.64), as well as between *UGT1A9*22* and *UGT1A7*3* (387T>G, 0.97; 622T>C, 0.46) was thought to be associated with toxicity profiles of each genotype. Therefore, the influence of not only genotypes but also haplotypes must be examined.[9,14,30] For haplotype I, which consists of all favorable alleles, there was a significant reduction in hematologic toxicity during the entire course of therapy ($P = 0.031$) as well as after the first cycle ($P = 0.080$). This protective haplotype was the most frequent haplotype (0.520) in Japanese patients.[9] In contrast, haplotype II, which contains four high-risk alleles (UGT1A1*6, *UGT1A7*3* [387G and 622C] and UGT1A9*1 [T9]), showed significantly higher hematologic toxicity during the entire course of therapy (haplotype frequency, 0.173; $P = 0.010$). The odds ratio for that toxicity was extremely high, and this haplotype was the second most common haplotype in Japanese patients, which differs considerably from that of Caucasians. This haplotype may be the leading cause of ethnic differences in irinotecan toxicity. Consequently, haplotype analysis seemed to have a stronger impact than each genotype analysis. Although toxicity was very severe in these patients, the response rate was not impaired.

There were some differences in profiles between first-line and second-line FOLFIRI treatments. In the first-line group, *UGT1A1*28* was a risk factor for severe hematological toxicity after the first cycle of FOLFIRI, and *UGT1A7* (387G) and *UGT1A9*22* (T9) genotypes were risk factors during the entire course of therapy. In the second-line group, older age was a risk factor after the first cycle, and *UGT1A7* (387G) and *UGT1A9* (T9) alleles and older age were risk factors during the entire course of therapy for severe hematological toxicity. In the second-line therapy group, older patients could not tolerate the sequential treatments. Objective tumor responses were predicted by *UGT1A1*6* and *UGT1A1*60*, but these results might be controversial.

Homozygosity of *UGT1A1*28* or **6* and compound heterozygosity of **28* and **6* have been reported as high-risk factors for hematologic toxicity, especially in Asia.[29] In our study, all **28/*6* and **6/*6* patients developed severe hematological toxicity. Moreover, one of two **28* homozygous patients suffered from severe hematological toxicity despite the reduced dosage of irinotecan (100 mg/m^2) used for these patients (Table S7). Although toxicity was very severe in these patients, the response rate was not impaired (3/7: 42.9%). For these patients, although the FOLFIRI regimen should be given in a careful manner, it is effective, and its use for advanced colorectal cancer patients should not be precluded from the treatment options.

In conclusion, assessment of *UGT1A1*28* and **6* and also *UGT1A7*3* and *UGT1A9*22* is very important to predict the toxicity of irinotecan in Japanese (or Asian) patients. Although each *UGT1A1*6* (211A), *UGT1A7*3* (387G), and *UGT1A9*22* (T9) allele might predict hematological toxicity, haplotype II (containing four risk alleles, *UGT1A1*6*, *UGT1A7*3* [387G and 622C], *UGT1A9*1* [T9]) and homozygosity of *UGT1A1*28* and **6* were better predictors of toxicity. For these patients, the FOLFIRI regimen should be given carefully. We will attempt further haplotype analysis by carrying out a new prospective study and using bioinformatics.

Acknowledgments

This study was supported in part by a non-profit organization Epidemiological and Clinical Research Information Network (ECRIN), as well as a Grant-in-Aid for Scientific Research of the Ministry of Education, Science, Sports and Culture of Japan (project No. 21591725 and 19591545). We thank Ms. Mai Hatta for her excellent clinical research coordination.

Disclosure Statement

The authors have no conflict of interest.

References

1 Douillard JY, Cunningham D, Roth AD *et al.* Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. *Lancet* 2000; **355**: 1041–7.

2 Saltz LB, Cox JV, Blanke C *et al.* Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. *N Engl J Med* 2000; **343**: 905–14.

3 Tournigand C, Andre T, Achille E *et al.* FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. *J Clin Oncol* 2004; **22**: 229–37.

4 Kawato Y, Aonuma M, Hirota Y, Kuga H, Sato K. Intracellular roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the antitumor effect of CPT-11. *Cancer Res* 1991; **51**: 4187–91.

5 Iyer L, King CD, Whitington PF *et al.* Genetic predisposition to the metabolism of irinotecan (CPT-11). Role of uridine diphosphate glucuronosyltransferase isoform 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes. *J Clin Invest* 1998; **101**: 847–54.

6 Beutler E, Gelbart T, Demina A. Racial variability in the UDP-glucuronosyltransferase 1 (UGT1A1) promoter: a balanced polymorphism for regulation of bilirubin metabolism? *Proc Natl Acad Sci U S A* 1998; **95**: 8170–4.

doi: 10.1111/cas.12283

7 Gagne JF, Montminy V, Belanger P, Journault K, Gaucher G, Guillemette C. Common human UGT1A polymorphisms and the altered metabolism of irinotecan active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38). *Mol Pharmacol* 2002; **62**: 608–17.
8 Yamanaka H, Nakajima M, Katoh M *et al.* A novel polymorphism in the promoter region of human UGT1A9 gene (UGT1A9*22) and its effects on the transcriptional activity. *Pharmacogenetics* 2004; **14**: 329–32.
9 Cecchin E, Innocenti F, D'Andrea M *et al.* Predictive role of the UGT1A1, UGT1A7, and UGT1A9 genetic variants and their haplotypes on the outcome of metastatic colorectal cancer patients treated with fluorouracil, leucovorin, and irinotecan. *J Clin Oncol* 2009; **27**: 2457–65.
10 Ando M, Ando Y, Sekido Y, Ando M, Shimokata K, Hasegawa Y. Genetic polymorphisms of the UDP-glucuronosyltransferase 1A7 gene and irinotecan toxicity in Japanese cancer patients. *Jpn J Cancer Res* 2002; **93**: 591–7.
11 Fujita K, Ando Y, Nagashima F *et al.* Genetic linkage of UGT1A7 and UGT1A9 polymorphisms to UGT1A1*6 is associated with reduced activity for SN-38 in Japanese patients with cancer. *Cancer Chemother Pharmacol* 2007; **60**: 515–22.
12 Han JY, Lim HS, Shin ES *et al.* Comprehensive analysis of UGT1A polymorphisms predictive for pharmacokinetics and treatment outcome in patients with non-small-cell lung cancer treated with irinotecan and cisplatin. *J Clin Oncol* 2006; **24**: 2237–44.
13 Lankisch TO, Schulz C, Zwingers T *et al.* Gilbert's Syndrome and irinotecan toxicity: combination with UDP-glucuronosyltransferase 1A7 variants increases risk. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 695–701.
14 Levesque E, Belanger AS, Harvey M *et al.* Refining the UGT1A haplotype associated with irinotecan-induced hematological toxicity in metastatic colorectal cancer patients treated with 5-fluorouracil/irinotecan-based regimens. *J Pharmacol Exp Ther* 2013; **345**: 95–101.
15 Ando Y, Saka H, Ando M *et al.* Polymorphisms of UDP-glucuronosyltransferase gene and irinotecan toxicity: a pharmacogenetic analysis. *Cancer Res* 2000; **60**: 6921–6.
16 Innocenti F, Vokes EE, Ratain MJ. Irinogenetics: what is the right star? *J Clin Oncol* 2006; **24**: 2221–4.
17 Okuyama Y, Hazama S, Nozawa H *et al.* Prospective phase II study of FOLFIRI for mCRC in Japan, including the analysis of UGT1A1 28/6 polymorphisms. *Jpn J Clin Oncol* 2011; **41**: 477–82.
18 Kato T, Okuyama Y, Nagata N *et al. Multicenter phase II studies of FOLFIRI with reduced starting dose of irinotecan in metastatic CRC patients with homozygous for UGT1A1*28/*6.* Presented at the ASCO Gastrointestinal Cancers Symposium; 14–17 Jan 2009, San Francisco, CA.
19 Therasse P, Arbuck SG, Eisenhauer EA *et al.* New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000; **92**: 205–16.
20 The Food and Drug Administration Center for Drug Evaluation and Research held in November 2004. [Cited 21 Jul 2005.] Available from URL: http://www.fda.gov/2004.
21 Hazama S, Nagashima A, Kondo H *et al.* Phase I study of irinotecan and doxifluridine for metastatic colorectal cancer focusing on the UGT1A1*28 polymorphism. *Cancer Sci* 2010; **101**: 722–7.
22 Wang L, Hirayasu K, Ishizawa M, Kobayashi Y. Purification of genomic DNA from human whole blood by isopropanol-fractionation with concentrated NaI and SDS. *Nucleic Acids Res* 1994; **22**: 1774–5.
23 Kobayashi M, Hazama S, Takahashi K *et al.* Is there diversity among UGT1A1 polymorphism in Japan? *World J Gastrointest Oncol* 2011; **4**: 170–5.
24 Guillemette C, Ritter JK, Auyeung DJ, Kessler FK, Housman DE. Structural heterogeneity at the UDP-glucuronosyltransferase 1 locus: functional consequences of three novel missense mutations in the human UGT1A7 gene. *Pharmacogenetics* 2000; **10**: 629–44.
25 The R Project for Statistical Computing. [Cited 20 May 2013.] Available from URL: http://www.r-project.org/.
26 Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; **21**: 263–5.
27 Akiyama Y, Fujita K, Nagashima F *et al.* Genetic testing for UGT1A1*28 and *6 in Japanese patients who receive irinotecan chemotherapy. *Ann Oncol* 2008; **19**: 2089–90.
28 Innocenti F, Undevia SD, Iyer L *et al.* Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. *J Clin Oncol* 2004; **22**: 1382–8.
29 Satoh T, Ura T, Yamada Y *et al.* Genotype-directed, dose-finding study of irinotecan in cancer patients with UGT1A1*28 and/or UGT1A1*6 polymorphisms. *Cancer Sci* 2011; **102**: 1868–73.
30 Martinez-Balibrea E, Abad A, Martinez-Cardus A *et al.* UGT1A and TYMS genetic variants predict toxicity and response of colorectal cancer patients treated with first-line irinotecan and fluorouracil combination therapy. *Br J Cancer* 2010; **103**: 581–9.

Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1. Minor allele frequency in Japanese patients with metastatic colorectal cancer.

Table S2. *UGT1A* polymorphisms and severe toxicity by univariate analysis in Japanese patients with metastatic colorectal cancer.

Table S3. *UGT1A* polymorphisms and severe toxicity and objective responses by univariate analysis (FLIGHT1) in Japanese patients with metastatic colorectal cancer.

Table S4. *UGT1A* polymorphisms and severe toxicity and objective responses by univariate analysis (FLIGHT2) in Japanese patients with metastatic colorectal cancer.

Table S5. Multivariable logistic regression analysis in Japanese patients with metastatic colorectal cancer.

Table S6. *UGT1A* genotypes and progression-free survival in Japanese patients with metastatic colorectal cancer.

Table S7. *UGT1A1*28* and *6 polymorphisms and toxicity/objective responses in Japanese patients with metastatic colorectal cancer.

Published Ahead of Print on September 9, 2013 as 10.1200/JCO.2013.50.3789
The latest version is at http://jco.ascopubs.org/cgi/doi/10.1200/JCO.2013.50.3789

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Sentinel Node Mapping for Gastric Cancer: A Prospective Multicenter Trial in Japan

Yuko Kitagawa, Hiroya Takeuchi, Yu Takagi, Shoji Natsugoe, Masanori Terashima, Nozomu Murakami, Takashi Fujimura, Hironori Tsujimoto, Hideki Hayashi, Nobunari Yoshimizu, Akinori Takagane, Yasuhiko Mohri, Kazuhito Nabeshima, Yoshikazu Uenosono, Shinichi Kinami, Junichi Sakamoto, Satoshi Morita, Takashi Aikou, Koichi Miwa, and Masaki Kitajima

Yuko Kitagawa, Hiroya Takeuchi, and Masaki Kitajima, Keio University School of Medicine; Yu Takagi, Tokyo Medical University, Tokyo; Shoji Natsugoe, Yoshikazu Uenosono, and Takashi Aikou, Kagoshima University Graduate School of Medical Science, Kagoshima; Masanori Terashima, Fukushima Medical University, Fukushima; Nozomu Murakami, Ishikawa Prefectural Central Hospital; Takashi Fujimura, Shinichi Kinami, and Koichi Miwa, Kanazawa University Hospital, Ishikawa; Hironori Tsujimoto, National Defense Medical College; Nobunari Yoshimizu, Saitama Social Insurance Hospital, Saitama; Hideki Hayashi, Graduate School of Medicine, Chiba University, Chiba; Akinori Takagane, Iwate Medical University, Iwate; Yasuhiko Mohri, Mie University Graduate School of Medicine, Mie; Kazuhito Nabeshima, Tokai University; Satoshi Morita, Yokohama City University Graduate School of Medicine, Kanagawa; and Junichi Sakamoto, Nagoya University Graduate School of Medicine, Aichi, Japan.

Published online ahead of print at www.jco.org on September 9, 2013.

Supported by Grants No. H16-14-14, H17-14-13, and H19-022 from the Ministry of Health, Labour and Welfare of Japan and Grant-in-Aid No. 13357012 from the Ministry of Education, Culture, Sports, Science, Technology, and Scientific Research of Japan.

Clinical trial information: University Hospital Medical Information Network Clinical Trials Registry UMIN000000476.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Corresponding author: Yuko Kitagawa, MD, FACS, Department of Surgery, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo, 160-8582, Japan; e-mail: kitagawa@a3.keio.jp

0732-183X/13/3199-1/$20.00

DOI: 10.1200/JCO.2013.50.3789

ABSTRACT

Purpose
Complicated gastric lymphatic drainage potentially undermines the utility of sentinel node (SN) biopsy in patients with gastric cancer. Encouraged by several favorable single-institution reports, we conducted a multicenter, single-arm, phase II study of SN mapping that used a standardized dual tracer endoscopic injection technique.

Patients and Methods
Patients with previously untreated cT1 or cT2 gastric adenocarcinomas < 4 cm in gross diameter were eligible for inclusion in this study. SN mapping was performed by using a standardized dual tracer endoscopic injection technique. Following biopsy of the identified SNs, mandatory comprehensive D2 or modified D2 gastrectomy was performed according to current Japanese Gastric Cancer Association guidelines.

Results
Among 433 patients who gave preoperative consent, 397 were deemed eligible on the basis of surgical findings. SN biopsy was performed in all patients, and the SN detection rate was 97.5% (387 of 397). Of 57 patients with lymph node metastasis by conventional hematoxylin and eosin staining, 93% (53 of 57) had positive SNs, and the accuracy of nodal evaluation for metastasis was 99% (383 of 387). Only four false-negative SN biopsies were observed, and pathologic analysis revealed that three of those biopsies were pT2 or tumors > 4 cm. We observed no serious adverse effects related to endoscopic tracer injection or the SN mapping procedure.

Conclusion
The endoscopic dual tracer method for SN biopsy was confirmed as safe and effective when applied to the superficial, relatively small gastric adenocarcinomas included in this study.

J Clin Oncol 31. © 2013 by American Society of Clinical Oncology

INTRODUCTION

Gastric cancer remains a major cause of cancer death throughout Asia. Although advances in multimodal approaches have significantly improved management of localized and resectable gastric cancer, gastrectomy with regional lymphadenectomy remains the mainstay of multimodal therapeutic strategies. Gastrectomy with D2 lymph node dissection (D2 gastrectomy) has become a standard surgical approach for resectable gastric cancer worldwide.[1-3] Although improved long-term results were reported after D2 gastrectomy in comparison with D1 gastrectomy, surgical morbidity after D2 gastrectomy remains significant, particularly in Western countries.[4] Furthermore, the incidence of regional lymph node metastasis is limited in patients with cT1 or T2N0 gastric cancer, whereas D2 gastrectomy seems to be an overly invasive surgery for patients with pN0 gastric cancer. Nevertheless, because of the limitations of the sensitivity of preoperative diagnostic imaging methods to detect pathologic metastasis in regional lymph nodes, D2 gastrectomy has become a standard procedure to ensure cure, even for clinically node-negative patients. Therefore, we hypothesized that sentinel node (SN) mapping offers a promising tool to resolve this issue.[5,6] SN mapping was applied to the upstaging of colorectal cancer as an initial clinical application in GI malignancies.[7]

Although there are controversial aspects regarding the application of SN mapping in gastric cancer, which has a relatively complicated lymphatic flow, several successful single-institution studies have been reported.[8-11] However, the indications and the procedures applied for SN mapping in these previous reports varied. Therefore, a prospective