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H. 知的財産権の出願・登録状況（予定含）

1．特許取得

なし

２．実用新案登録
なし
３．その他
なし

Dose-dense paclitaxel once a week in combination with carboplatin every 3 weeks for advanced ovarian cancer: a phase 3, open-label, randomised controlled trial

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Summary

Background Paclitaxel and carboplatin given every 3 weeks is standard treatment for advanced ovarian carcinoma. Attempts to improve patient survival by including other drugs have yielded disappointing results. We compared a conventional regimen of paclitaxel and carboplatin with a dose-dense weekly regimen in women with advanced ovarian cancer.

Methods Patients with stage II to IV epithelial ovarian cancer, fallopian tube cancer, or primary peritoneal cancer were eligible for enrolment in this phase 3, open-label, randomised controlled trial at 85 centres in Japan. Patients were randomly assigned by computer-generated randomisation sequence to receive six cycles of either paclitaxel (180 mg/m^2; 3-h intravenous infusion) plus carboplatin (area under the curve [AUC] 6 mg/mL per min), given on day 1 of a 21-day cycle (conventional regimen; n=320), or dose-dense paclitaxel (80 mg/m^2; 1-h intravenous infusion) given on days 1, 8, and 15 plus carboplatin given on day 1 of a 21-day cycle (dose-dense regimen; n=317). The primary endpoint was progression-free survival. Analysis was by intention to treat (ITT). This trial is registered with ClinicalTrials.gov, number NCT00226915.

Findings 631 of the 637 enrolled patients were eligible for treatment and were included in the ITT population (dose-dense regimen, n=312; conventional regimen, n=319). Median progression-free survival was longer in the dose-dense treatment group (28·0 months, 95% CI 22·3–35·4) than in the conventional treatment group (17·2 months, 15·7–21·1; hazard ratio [HR] 0·71; 95% CI 0·58–0·88; p=0·0015). Overall survival at 3 years was higher in the dose-dense regimen group (72·1%) than in the conventional treatment group (65·1%; HR 0·75, 0·57–0·98; p=0·03). 165 patients assigned to the dose-dense regimen and 117 assigned to the conventional regimen discontinued treatment early. Reasons for participant dropout were balanced between the groups, apart from withdrawal because of toxicity, which was higher in the dose-dense regimen group than in the conventional regimen group (n=113 *vs* n=69). The most common adverse event was neutropenia (dose-dense regimen, 286 [92%] of 312; conventional regimen, 276 [88%] of 314). The frequency of grade 3 and 4 anaemia was higher in the dose-dense treatment group (214 [69%]) than in the conventional treatment group (137 [44%]; p<0·0001). The frequencies of other toxic effects were similar between groups.

Interpretation Dose-dense weekly paclitaxel plus carboplatin improved survival compared with the conventional regimen and represents a new treatment option in women with advanced epithelial ovarian cancer.

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Introduction

Paclitaxel and carboplatin given every 3 weeks is currently considered standard first-line chemotherapy for advanced epithelial ovarian cancer. The consensus statements on the management of ovarian cancer at the 3rd International Gynecologic Cancer Consensus Conference in 2004 recommended intravenous paclitaxel (175 mg/m^2 over 3 h) plus intravenous carboplatin (area under the curve [AUC] 5·0–7·5 mg/mL per min) given every 3 weeks for six cycles for first-line chemotherapy.[1] Paclitaxel and carboplatin have been combined with other drugs, given either concurrently or sequentially, in the hope of prolonging survival in women with advanced ovarian cancer, but the results of several randomised trials have been disappointing.[2–4] In particular, the recently reported randomised trial of the Gynecologic Oncology Group, an international collaborative study enrolling more than 4500 patients, showed that the addition of new cytotoxic drugs to paclitaxel plus carboplatin did not improve progression-free or overall survival.[2]

Dose-dense weekly administration of paclitaxel is another strategy to enhance antitumour activity and prolong survival. Preclinical studies have suggested that duration of exposure is an important determinant of the cytotoxic activity of paclitaxel.[5] Adequate cytotoxicity can be achieved at fairly low concentrations of the drug provided that exposure is extended.[5,6] Several phase 2 clinical trials of dose-dense weekly paclitaxel and carboplatin have shown promising efficacy and favourable tolerability in women with ovarian cancer.[7–9]

We undertook a phase 3, randomised controlled trial to compare conventional paclitaxel and carboplatin given every 3 weeks with dose-dense paclitaxel given every week plus carboplatin (every 3 weeks) as first-line treatment in women with advanced ovarian cancer.

Methods

Patients

Patients from 85 centres in Japan were eligible for enrolment in this phase 3, open-label, randomised trial if they had a histologically or cytologically proven diagnosis of stage II to IV epithelial ovarian cancer, fallopian tube cancer, or primary peritoneal cancer. If only the results of cytological examinations were available, patients needed to have the following criteria: (1) a cytological diagnosis of adenocarcinoma; (2) an abdominal mass more than 2 cm in diameter on abdominal images; and (3) a CA125/carcinoembryonic antigen (CEA) ratio[10] of more than 25, or no evidence of gastrointestinal cancer if CA125/CEA ratio was less than or equal to 25. Previous chemotherapy was not allowed. Patients needed to be aged 20 years or older, to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0–3,[11] and to have adequate organ functions, defined as absolute neutrophil count 1·5 cells$\times10^9$ per L or more, platelet count 100×10^9 per L or more, serum bilirubin 25·7 μmol/L or less, serum aspartate aminotransferase 100 IU/L or less, and serum creatinine 132·6 μmol/L or less. Patients were excluded if they had an ovarian tumour with a low malignant potential, or synchronous or metachronous (within 5 years) malignant disease other than carcinoma in situ.

All patients gave written informed consent before enrolment in this study. The study protocol was approved by the institutional review boards at all participating centres. The protocol was coordinated by the Japanese Gynecologic Oncology Group (protocol number 3016).

Randomisation and masking

Patients were randomly assigned to receive paclitaxel and carboplatin in either a conventional regimen (control) or a dose-dense regimen (intervention). Randomisation was by telephone or fax from a central registration centre located at University of Toyama (Toyama, Japan), and the random allocation table was computer-generated by use of the SAS PROC PLAN. Randomisation was stratified by residual disease (≤1 cm *vs* >1 cm), International Federation of Gynecology and Obstetrics (FIGO) stage (II *vs* III *vs* IV),[12] and histological type (clear-cell or mucinous tumours *vs* serous or other tumours), with adequate balancing within each institution. Patients and clinicians were not masked to treatment assignment.

Procedures

Both study groups received carboplatin at a dose calculated to produce an AUC of 6 mg/mL per min on day 1 of a 21-day cycle. Carboplatin was given as an intravenous infusion over 1 h. The control group also received paclitaxel given as a 3-h intravenous infusion at a dose of 180 mg/m^2 on day 1. In the dose-dense group, paclitaxel was given as a 1-h intravenous infusion at a dose of 80 mg/m^2 on days 1, 8, and 15. The dose of carboplatin was calculated with the formula of Calvert and colleagues,[13] by use of creatinine clearance instead of glomerular filtration rate. Creatinine clearance was calculated with the formula of Jelliffe.[14] Standard premedication was given to prevent hypersensitivity reactions to paclitaxel. The treatments were repeated every 3 weeks for six cycles. Patients with measurable lesions who had a partial response or complete response received three additional cycles of chemotherapy.

Patients needed to have an absolute neutrophil count of $1\cdot0\times10^9$ cells per L (amended from $1\cdot5\times10^9$ cells per L on April 11, 2005, because of frequent occurrence of delaying) or more and a platelet count of 75×10^9 per L or more to receive subsequent cycles of therapy in both groups. Patients in the dose-dense regimen group also had to have an absolute neutrophil count of $0\cdot5\times10^9$ cells per L or more and a platelet count of 50×10^9 per L (amended from 75×10^9 per L on April 11, 2005) or more before they received paclitaxel on days 8 and 15. Treatment was delayed for a maximum of 3 weeks (amended from 2 weeks on April 11, 2005).

The dose of carboplatin was reduced for haematological toxicity, and paclitaxel was reduced for non-haematological toxicity with dose reduction levels as follows: carboplatin AUC 5 mg/mL per min (level 1) or AUC 4 mg/mL per min (level 2) in both groups; paclitaxel 135 mg/m^2 (level 1) or 110 mg/m^2 (level 2) in the conventional treatment group, and paclitaxel 70 mg/m^2 (level 1) or 60 mg/m^2 (level 2) in the dose-dense treatment group. The carboplatin dose was reduced when febrile neutropenia occurred, an absolute neutrophil count less than $0\cdot5\times10^9$ cells per L persisted for 7 days or more, the platelet count was less than 10×10^9 per L, the platelet count was between 10×10^9 per L and 50×10^9 per L with bleeding tendencies, or the treatment was delayed for haematological toxicity for more than 1 week. In general, patients did not receive prophylactic granulocyte-colony stimulating factor (G-CSF) unless they had treatment delays or neutropenic complications after treatment. The dose of paclitaxel was reduced in patients who had grade 2 or higher peripheral neuropathy.

Interval debulking surgery after two to four cycles of chemotherapy, secondary debulking or second-look surgery after six cycles of chemotherapy, or both, were allowed. These procedures were done within 6 weeks after chemotherapy, and subsequent chemotherapy was restarted within 6 weeks after surgery.

The primary endpoint of this trial was progression-free survival, defined as the time from the date of randomisation to the date of the first occurrence of any of the following events: death from any cause; appearance of any new lesions that could be measured or assessed clinically;

or CA125 criteria of disease progression.[15] The CA125 criteria of disease progression were defined as (1) patients with raised CA125 concentration before treatment with a return to normal after treatment needed to show re-elevation of CA125 greater than or equal to two times the upper normal limit; (2) patients with raised CA125 before treatment that did not return to normal needed to show evidence of CA125 greater than or equal to two times the nadir value; or (3) patients with CA125 in the normal range before treatment needed to show evidence of CA125 greater than or equal to two times the upper normal limit, with raised CA125 recorded on two occasions at least 1 week apart. In patients with measurable disease, clinical or radiographical tumour measurements had priority over CA125 concentration, and progression during treatment could not be declared on the basis of CA125 alone.

Secondary endpoints were overall survival, response rate, and adverse events. The planned analyses of progression-free survival and overall survival included data on eligible patients according to the intention-to-treat (ITT) principle. Clinical response was assessed in eligible patients with lesions that could be measured in two dimensions. The assessment of response had to be confirmed on two occasions at least 4 weeks apart. A complete response was defined as the complete disappearance of all measurable and assessable lesions, determined by two observations not less than 4 weeks apart. A partial reponse was defined as a 50% or greater decrease in the sum of the products of the perpendicular diameters of measurable lesions, determined by two observations not less than 4 weeks apart. Stable disease was defined as a steady state of response less than a partial response or as an increase of less than 25% in the sum of the products of the perpendicular diameters of measurable lesions, lasting at least 4 weeks. Progressive disease was defined as an unequivocal increase of at least 25% in the sum of the products of the perpendicular diameters of measurable lesions. The appearance of new lesions also constituted progressive disease. Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria version 2.0.[16]

Radiological studies to record the status of all measurable lesions noted at baseline were repeated after two, four, and six cycles of chemotherapy. Once patients discontinued the protocol therapy, disease status was assessed every 3 months for the first 2 years and every 6 months thereafter. Follow-up monitoring included clinical examinations and CA125 concentration estimation; routine CT scans were not required, but were requested if CA125 concentration rose, symptoms of relapse developed, or both.

Statistical analysis

Our hypothesis was that the dose-dense regimen would prolong progression-free survival compared with the conventional regimen. At the beginning of the study in April, 2003, a sample size of 380 patients with no interim analysis was initially planned to detect a 37·5% improvement in median progression-free survival in the conventional regimen group (from 16 months to 22 months) with 80% power, two-sided log-rank test, and alpha level of 0·05. In January, 2005, the sample size was increased to 600 patients during the trial to account for the higher accrual of patients and to detect a shorter prolongation of progression-free survival. This amendment of the protocol was made without interim analysis and was approved by the data and safety monitoring committee. The increased sample size would enable the detection of a 31·3% improvement (from 16 months to 21 months) in median progression-free survival with 80% power, two-sided log-rank test, at an alpha level of 0·05, an accrual of 3 years, and a follow-up of 1·5 years. Following the data safety monitoring committee's instructions, interim analysis was planned after 380 patients had been randomly assigned to treatment, and multiplicity by multiple look was adjusted with the

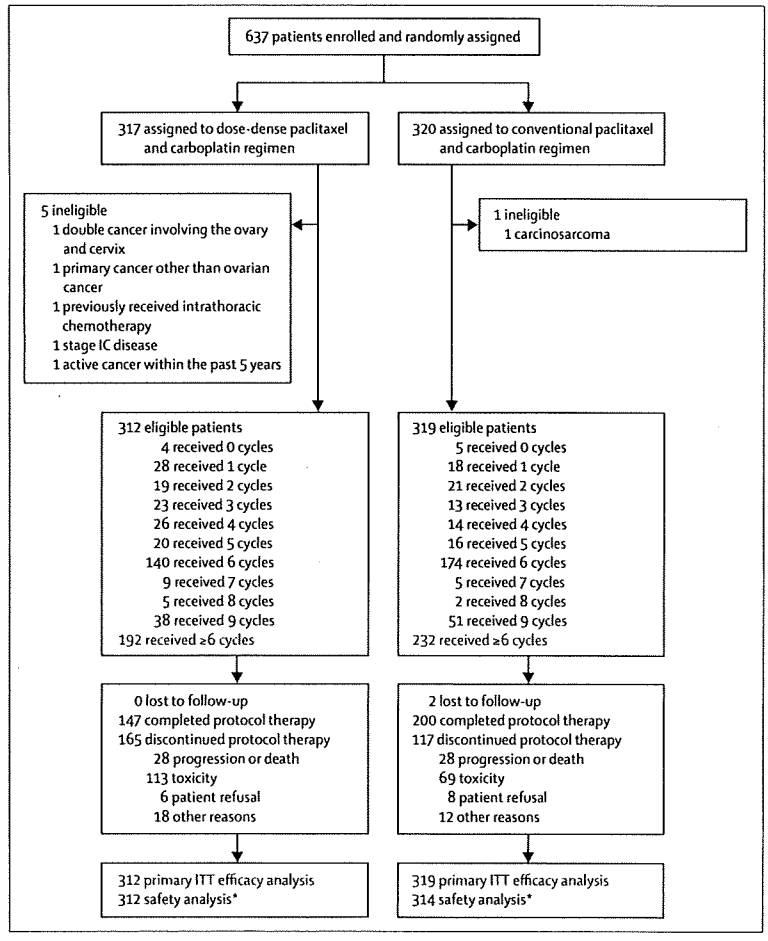

Figure 1: **Trial profile**
ITT=intention-to-treat. *Analysis of safety includes all randomised women who had received at least one cycle of treatment (one ineligible patient in each group did not receive treatment).

	Dose-dense regimen group (n=312)	Conventional regimen group (n=319)
Age (years)	57 (25–87)	57 (25–84)
FIGO stage		
II	62 (20%)	54 (17%)
III	202 (65%)	215 (67%)
IV	48 (15%)	50 (16%)
ECOG performance status		
0 or 1	283 (91%)	287 (90%)
2	23 (7%)	20 (6%)
3	6 (2%)	12 (4%)
Disease		
Ovarian	260 (83%)	276 (87%)
Fallopian tube	14 (4%)	18 (6%)
Primary peritoneal	38 (12%)	25 (8%)
Surgery		
Cytology only	35 (11%)	35 (11%)
Primary debulking	277 (89%)	284 (89%)
Interval debulking	34 (11%)	29 (9%)
Secondary/second-look	38 (12%)	56 (18%)
Residual disease		
≤1 cm	144 (46%)	145 (45%)
>1 cm	168 (54%)	174 (55%)
Histological type		
Serous adenocarcinoma	173 (55%)	182 (57%)
Endometrioid adenocarcinoma	38 (12%)	39 (12%)
Clear-cell carcinoma	31 (10%)	37 (12%)
Mucinous adenocarcinoma	23 (7%)	11 (3%)
Other types	47 (15%)	50 (16%)
Histological grade		
Well differentiated	42 (13%)	40 (13%)
Moderately differentiated	60 (19%)	71 (22%)
Poorly differentiated	79 (25%)	72 (23%)
Unknown/not applicable	131 (42%)	136 (43%)

Data are n (%) or median (range). FIGO=International Federation of Gynecology and Obstetrics. ECOG=Eastern Cooperative Oncology Group.

Table 1: **Baseline characteristics of study patients**

O'Brien-Fleming alpha-spending function. At the first interim analysis in December, 2005, the data safety monitoring committee reviewed the results and approved continuation of the planned follow-up.

The cumulative survival curve and median progression-free survival time were estimated by use of the Kaplan-Meier method. Adverse events were analysed in all randomised women who had received at least one cycle of treatment. Proportions of adverse events were compared between the groups by the use of two-sided χ^2 tests or two-sided Fisher's exact tests. Responses were compared by the use of Fisher's exact test. All analyses were performed with SAS software, version 8.2. This trial is registered with ClinicalTrials.gov, number NCT00226915.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between April, 2003, and December, 2005, 637 patients were enrolled at 85 centres. Figure 1 shows the trial profile. Table 1 shows the baseline characteristics of the 631 eligible patients whose data were included in the ITT analysis.

The median number of treatment cycles was six in both groups (figure 1). The proportion of patients who received six or more cycles of treatment was higher in the conventional regimen group (232 [73%] of 319) than in the dose-dense regimen group (192 [62%] of 312). The main reason for discontinuing treatment was toxicity. Haematological toxicity was the most common form of toxicity leading to the discontinuation of treatment (68 [60%] of 113 patients assigned to the dose-dense regimen *vs* 30 [43%] of 69 assigned to the conventional regimen; p=0·03). The proportions of patients who discontinued treatment because of neurotoxicity were low in both groups (three [3%] *vs* five [7%]). Other reasons for discontinuation of treatment because of toxic effects were patient refusal (13 [12%] *vs* 12 [17%]), allergic reaction (four [4%] *vs* seven [10%]), and other toxic effects (25 [22%] *vs* 15 [22%]).

At least one treatment cycle was delayed in a higher proportion of patients in the dose-dense treatment group (236 [76%] of 312) than in the conventional treatment group (213 [67%] of 319; p=0·02). The dose of the study drugs was reduced in a higher proportion of patients assigned to the dose-dense regimen (150 [48%] of 312) than in those assigned to the conventional regimen (112 [35%] of 319; p=0·001). The mean delivered dose intensity of carboplatin was lower in the dose-dense regimen group (AUC per week 1·54 mg/mL per min [SD 0·37]) than in the conventional regimen group (AUC per week 1·71 mg/mL per min [SD 0·36]), and the mean delivered dose-intensity of paclitaxel was higher (63·0 mg/m² per week [SD 13·0] *vs* 51·7 mg/m² per week [SD 10·6]). The mean relative dose intensities of carboplatin and paclitaxel were both lower in the dose-dense regimen group (77% [SD 18] and 79% [SD 15], respectively) than in the conventional regimen group (85% [SD 18], and 86% [SD 18], respectively).

At the time of last follow-up (December, 2007), with a median duration of follow-up of 29 months, there had been 160 disease progression events in the dose-dense treatment group and 200 in the conventional treatment group. Median progression-free survival was 28·0 months (95% CI 22·3–35·4) in the dose-dense treatment group and 17·2 months (15·7–21·1) in the

conventional treatment group (figure 2; unadjusted hazard ratio [HR] 0·71, 95% CI 0·58–0·88; p=0·0015, log-rank test). When the analysis was done with data from all 637 patients who were randomly assigned to treatment, the result was similar (p=0·0019). After adjustment for FIGO stage, residual disease, and histological type according to the preplanned analysis, the HR was 0·65 (0·53–0·80; p=0·0001). We subsequently undertook unplanned sensitivity analyses. The differences between groups were still significant when only clinical progression was defined as progression (p=0·0018), when data on patients who received second-line therapy before progression were censored (dose-dense regimen, n=3; conventional regimen, n=5; p=0·0018), or when data on patients who underwent interval or secondary surgery, or both, were censored (dose-dense regimen, n=71; conventional regimen, n=85; p=0·0092).

Analysis of overall survival was done in December, 2007, at the same time as the analysis of progression-free survival. The overall survival at 2 years was 83·6% in the dose-dense treatment group and 77·7% in the conventional treatment group (p=0·049). We updated the overall survival analysis in December, 2008, with median follow-up period of 42 months. Although median overall survival had not been reached in either group, overall survival at 3 years was higher in the dose-dense treatment group (72·1%) than in the conventional treatment group (65·1%; unadjusted HR 0·75, 0·57–0·98; p=0·03 log-rank test; figure 2).

A Cox proportional-hazards model was used to examine the effect of baseline clinical characteristics and conventional prognostic factors on the treatment effect (figure 3). Progression-free survival was longer in the dose-dense treatment group than in the conventional treatment group across all subgroups of patients apart from in those with clear-cell or mucinous tumours. In this subgroup of patients, the HR in the dose-dense treatment group was similar to that in the conventional treatment group.

Clinical response was assessed in 282 patients who had measurable disease at study entry. The overall response rate was similar between groups (conventional regimen, 72 [53%] of 135 patients; dose-dense regimen, 82 [56%] of 147 patients; p=0·72; table 2). Because patients who underwent suboptimally debulked surgery (>1 cm of residual disease) were allowed to undergo interval debulking surgery in this study, response sometimes could not be confirmed on repeated imaging. If these unconfirmed responses are taken into account (44 patients), the overall response rate was 70% (94 of 135 patients) in the conventional treatment group compared with 71% (104 of 147 patients) in the dose-dense treatment group (p=0·90).

Treatment-related adverse events were analysed in patients who received at least one cycle of the study treatment (table 3). The frequency of grade 3 or 4 anaemia was higher in the dose-dense treatment group than in the conventional treatment group (p<0·0001). Recombinant erythropoietin was not used to treat anaemia because it was not approved in Japan. G-CSF was used in 187 (60%) patients assigned to the dose-dense regimen and in 214 (67%) assigned to the conventional regimen. The frequency of neuropathy did not differ between study groups.

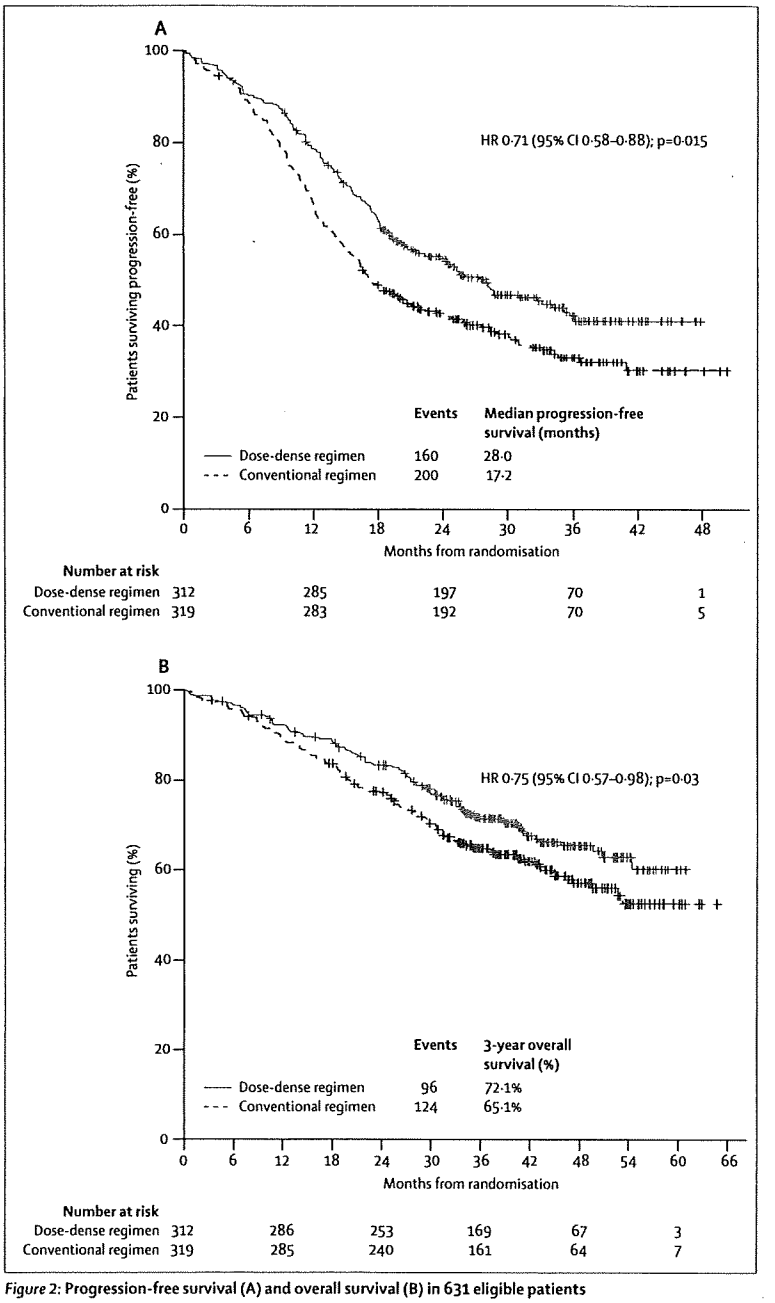

Figure 2: **Progression-free survival (A) and overall survival (B) in 631 eligible patients**
HR=hazard ratio.

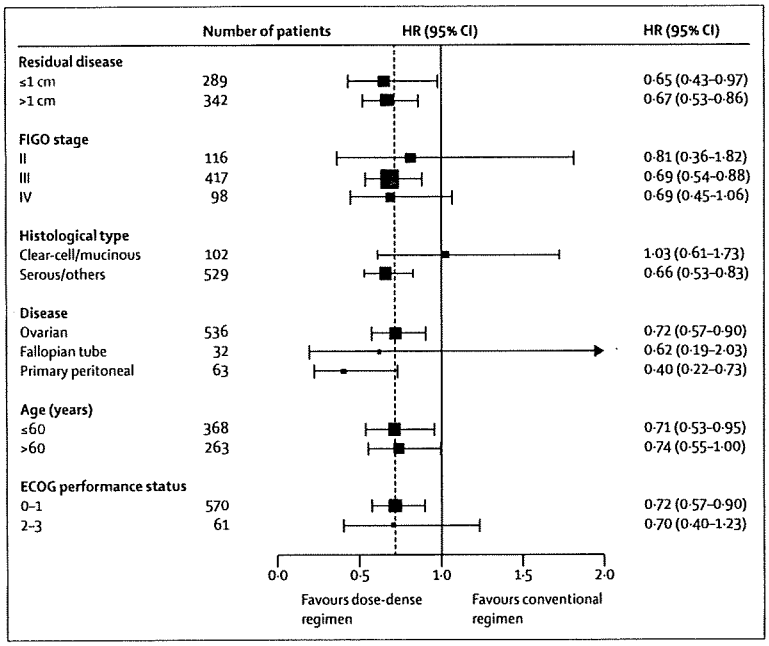

Figure 3: **Progression-free survival according to baseline characteristics**
FIGO=International Federation of Gynecology and Obstetrics. ECOG=Eastern Cooperative Oncology Group. The hazard ratios (HRs; 95% CIs) are for patients assigned to conventional paclitaxel and carboplatin, compared with those assigned to dose-dense paclitaxel and carboplatin, and were obtained from the unadjusted Cox model. The dashed vertical line indicates a hazard ratio of 0·71, which is the value for all patients, and the solid vertical line indicates a hazard ratio of 1·00, which is the null-hypothesis value.

	Dose-dense regimen group (n=147)	Conventional regimen group (n=135)	p value
Complete response	29 (20%)	21 (16%)	0·44
Partial response	53 (36%)	51 (38%)	0·81
Stable disease	43 (29%)	42 (31%)	0·80
Progressive disease	4 (3%)	9 (7%)	0·16
Not evaluable	18 (12%)	12 (9%)	0·44

See Methods section for definitions of responses.

Table 2: **Clinical response in patients with measurable lesions**

	Dose-dense regimen group (n=312)	Conventional regimen group (n=314)	p value
Neutropenia	286 (92%)	276 (88%)	0·15
Thrombocytopenia	136 (44%)	120 (38%)	0·19
Anaemia	214 (69%)	137 (44%)	<0·0001
Febrile neutropenia	29 (9%)	29 (9%)	1·00
Nausea	32 (10%)	36 (11%)	0·70
Vomiting	9 (3%)	11 (4%)	0·82
Diarrhoea	10 (3%)	8 (3%)	0·64
Fatigue	15 (5%)	8 (3%)	0·14
Arthralgia	3 (1%)	5 (2%)	0·72
Myalgia	2 (1%)	4 (1%)	0·69
Neuropathy (motor)	15 (5%)	12 (4%)	0·56
Neuropathy (sensory)	21 (7%)	20 (6%)	0·87

Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria version 2.0.[16]

Table 3: **Frequency of grade 3 or 4 adverse events**

Discussion

Our study showed that compared with a conventional regimen, dose-dense treatment with paclitaxel and carboplatin improved progression-free survival in women with newly diagnosed, stage II to IV ovarian cancer. Women assigned to dose-dense paclitaxel and carboplatin had a 29% lower risk of disease progression and a 25% lower risk of death than did patients assigned to the conventional regimen. Benefits of this magnitude have been rare in women with advanced ovarian cancer, including those with suboptimally debulked stage III and IV disease, since the approval of paclitaxel for the indication of ovarian cancer.

The concept of dose density is based on the hypothesis that a shorter interval between doses of cytotoxic therapy would more effectively reduce tumour burden than would dose escalation.[17] In breast cancer, recently published phase 3 trials have shown that paclitaxel given every week improves response and survival.[18,19] Consistent with these findings, our study showed that progression-free survival and overall survival were significantly longer in the dose-dense regimen group than in the conventional regimen group. Increased doses of paclitaxel of 225 mg/m² or 250 mg/m² given every 3 weeks have been compared with the standard dose (ie, 175 mg/m²) in women with ovarian cancer, but showed no benefit in survival.[20,21] Our study showed a survival advantage with an increased total dose of 240 mg/m², given in three divided doses during a 21-day cycle, suggesting that dose density is more important than increased dose intensity.

There was greater haematological toxicity in the dose-dense treatment group than in the conventional treatment group, which resulted in more delays and dose modifications. The optimum dose and schedule of dose-dense paclitaxel and carboplatin have not yet been established. Rose and colleagues[8] reported that weekly paclitaxel at a dose of 60 mg/m² in combination with carboplatin at an AUC of 5 mg/mL per min was tolerated and active in patients with recurrent ovarian cancer. An alternative schedule of dose-dense treatment is to give both paclitaxel and carboplatin every week. Sehouli and co-workers[9] showed that weekly paclitaxel at a dose of 100 mg/m² and weekly carboplatin at an AUC of 2 mg/mL per min showed substantial activity and tolerability in patients with primary ovarian cancer. A treatment delay occurred in only 2·8% of cycles and the frequency of grade 3 neurotoxicity (2% [three of 129 patients]) was lower than that reported in our study. Additionally, weekly carboplatin of AUC 2 mg/mL per min and weekly paclitaxel of 60 mg/m² on days 1, 8, and

15 every 4 weeks showed a favourable toxicity profile in elderly ovarian cancer patients.[22]

The response rate did not differ between groups. Virtually all previous randomised trials in ovarian cancer that showed an improvement in progression-free survival and overall survival also had a higher response rate for the more effective treatment. A lower dose of paclitaxel had antiangiogenic activity in a xenograft model.[23] Antiangiogenic agents might promote tumour dormancy by maintaining tumour size and preventing outgrowth.[24] Vascular endothelial growth factor (VEGF) is frequently expressed in ovarian cancer, and might be an important therapeutic target. Longer survival in the dose-dense regimen group without an improved response rate might be attributed to the antiangiogenic effect of paclitaxel. Anti-VEGF agents such as bevacizumab combined with the dose-dense treatment will be assessed in future trials.

Neurotoxicity is the adverse reaction of greatest concern in patients who receive a combination of paclitaxel and carboplatin. In breast cancer trials, the incidence of neurotoxicity was higher in patients given paclitaxel every week than in patients given paclitaxel every 3 weeks.[19] In our study, however, the frequency of neurotoxicity was similar in both groups. This finding might be because patients in the dose-dense treatment group discontinued treatment more often than did those in the conventional treatment group.

Fewer than half the patients assigned to the dose-dense regimen completed treatment according to the study protocol. When designing the protocol, we debated whether patients who responded to six cycles of chemotherapy should receive three more cycles. However, this study was not designed to assess the relation between the duration of treatment and clinical outcomes, and there is little evidence to suggest that more than six cycles of chemotherapy would prolong survival. About 60% of patients in the dose-dense regimen group received six or more cycles of chemotherapy. Treatment cycles were more frequently delayed in the dose-dense treatment group than in the conventional treatment group, mainly because of neutropenia.

Clear-cell and mucinous adenocarcinoma of the ovary is associated with low sensitivity to chemotherapy and poor survival.[25,26] In our study, neither dose-dense nor conventional treatment seemed effective against clear-cell or mucinous ovarian carcinoma, which suggests that other treatment strategies are needed.

Thus, our study showed that a dose-dense regimen of paclitaxel once a week plus carboplatin every 3 weeks is associated with longer progression-free and overall survival than a conventional regimen of paclitaxel and carboplatin given every 3 weeks in women with advanced epithelial ovarian cancer.

Contributors

NK, MY, FT, SI, TS, EK, and KO conceived and designed the study with the Japanese Gynecologic Oncology Group. MY was the coordinating principal investigator for the study. NK and FT analysed and interpreted the results. NK drafted the report. KN was responsible for the overall planning and conduct of the study. NK, MY, SI, TJ, DA, HT, TS, SK, EK, and KO were involved in the provision of study material or patients, or data acquisition. NK, MY, TS, EK, and KO were members of the steering committee. All authors were involved in writing the report and approved the final version of the manuscript.

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Conflicts of interest

SI and DA have received honoraria from Bristol-Myers Squibb. DA and HT have received grant support from Bristol-Myers Squibb. All other authors declare that they have no conflicts of interest.

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Carbonic anhydrase IX and human papillomavirus as diagnostic biomarkers of cervical dysplasia/neoplasia in women with a cytologic diagnosis of atypical glandular cells: A Gynecologic Oncology Group study in United States

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High-risk human papillomavirus (H-HPV) infection is strongly linked to cervical neoplasia, but its role in detecting glandular lesions (GLs) is unclear. In the cervix, carbonic anhydrase IX (CA-IX) is expressed in cervical neoplasia, but rarely in the benign cervix. The diagnostic utility of these biomarkers was evaluated in women with a cytologic diagnosis of atypical glandular cells (AGC). H-HPV was detected using hybrid capture 2 (HC2) in liquid-based cytology, and CA-IX immunoreactivity was studied on conventional Pap smears. Of 403 patients, 111 (28%) were positive for significant cervical lesions (SCLs) including CIN2, CIN3, adenocarcinoma *in situ* or invasive carcinoma. CA-IX testing alone (n = 403) had a sensitivity of 75, 95 or 65% for SCLs, significant GLs or squamous lesions (SLs), respectively, with a specificity of 88% and a false negative rate (FNR defined as 1 minus negative predictive value) of 10%. Testing for H-HPV (n = 122) had a sensitivity of 97, 100 or 96% for SCLs, GLs or SLs, respectively, with a specificity of 87% and a FNR of 1%. The combination of CA-IX and H-HPV testing (n = 122), collectively, had the same sensitivity, specificity and FNR for SCLs, GLs or SLs as H-HPV testing alone. The conclusions of our study are that both H-HPV and CA-IX testing are useful diagnostic markers for GLs. However, H-HPV testing is a better diagnostic marker for SLs. The combination of CA-IX with H-HPV testing does not improve the diagnostic accuracy for cervical neoplasia in women with AGC diagnosis over that of H-HPV testing alone.
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Key words: CA-IX; HPV; AGC diagnosis; cervix

In the United States (U.S.), an estimated 11,070 new cases of cervical cancers will be diagnosed and 3,870 women will die from cervical cancer in 2008.[1] Although a majority of these cases are squamous lesions, the proportion of adenocarcinomas relative to squamous cell carcinomas is increasing, and current cervical cytologic screening fails to detect a significant proportion of glandular lesions (GLs).[2]

Unlike squamous lesions, the cytologic criteria for identifying glandular neoplasia are not well established. To better classify these abnormalities, the term atypical glandular cells of undetermined significance (AGUS) was introduced in the 1988 Bethesda System (TBS). In 1991, AGUS was qualified according to the possible anatomic site of origin, endocervical *versus* endometrial.[3] The 2001 TBS replaced AGUS with the term atypical glandular cells (AGC) and classified glandular cell abnormalities less severe than adenocarcinoma into 3 categories: AGC of unclear cell origin, atypical endocervical cells (AEC) and atypical endometrial cells (AEMC).[4] However, in clinical practice, subcategorization of the AGC, particularly in the category of AGC/AEC, remains a diagnostic challenge with poor interobserver agreement.[5] In patients with AGC, the rates of CIN2 or CIN3 (high-grade squamous intraepithelial lesion or HSIL) and adenocarcinoma *in situ* (AIS) range from 5 to 50% and 0 to 15%, respectively, with rates of invasive carcinoma of up to 10%.[6–9] The Society of Gynecologic Oncologists has urged more aggressive evaluation of patients with a diagnosis of AGC because of the relatively high (mean, 41%) proportion of women who eventually are found to harbor high-grade cervical neoplastic lesions.[9]

The clinical management of AGC is also limited by the relative lack of accuracy of colposcopy and endocervical curettage for excluding cervical glandular neoplasia. Significant lesions, including invasive carcinoma, may exist in patients after negative colposcopy and endocervical sampling.[10] Although AGC diagnosis only represents a small fraction (~0.5%) of total Pap smear diagnoses, its association with significant underlying cervical lesions has posed a particular dilemma in clinical management, both from a cost-benefit standpoint and from a desire to avoid unnecessary invasive procedures.[11,12] An accurate screening method or test is needed to determine which women with a cytologic diagnosis of AGC harbor a significant cervical lesion.

In the 1990s, the antigen MN was identified.[13] MN is a transmembrane glycoprotein, is a member of the carbonic anhydrase gene family and has been given the designation, carbonic anhydrase IX (CA-IX).[14] CA-IX is a biomarker of several human tumors, including carcinomas of the cervix and kidney.[15,16] CA-

Abbreviations: AEC, atypical endocervical cells; AEMC, atypical endometrial cells; AGC, atypical glandular cells; AGUS, atypical glandular cells of undetermined significance; AIS, adenocarcinoma *in situ*; CA-IX, carbonic anhydrase IX; FNR, false-negative rate defined as 1 − NPV to reflect the proportion of negative diagnoses that were incorrect; GLs, significant glandular lesions; GOG, gynecologic oncology group; H-HPV, high-risk human papillomavirus; HC2, hybrid capture II; HSIL, high-grade squamous intraepithelial lesion; LBC, liquid-based cytology; NPV, negative predictive value; PPV, positive predictive value; RLA, Roche linear array; SAS, statistical analysis system; SCL, significant cervical lesion; SLs, significant squamous lesions; TBS, the Bethesda System; U.S., United States.

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IX expression in cancerous tissues, and its absence in normal counterparts, has led to the speculation that it plays a role in carcinogenesis.[17] Its expression is controlled by the transcription factor, hypoxia inducible factor-1 (HIF-1), and is upregulated in hypoxic regions of tumor tissues. CA-IX expression has been associated with a poor prognosis in cervical and breast cancers.[18,19]

In a survey of benign and neoplastic cervical tissues and Pap smears, it was observed that virtually all AGC associated with AIS and adenocarcinoma expressed high levels of CA-IX antigen, whereas endocervical cells derived from benign cervical tissues were negative, suggesting that CA-IX protein would be a useful biomarker for diagnosing AIS and invasive adenocarcinoma.[6,15,20]

Infection with human papillomavirus (HPV) is an etiologic factor for both glandular and squamous cervical cancer. HPV has been identified in 80–90% of adenocarcinomas and their precursor lesions; however, there are only limited data regarding the role of HPV testing in the detection of glandular neoplasia.[12,21–26]

Thus, the Gynecologic Oncology Group (GOG), a national multiinstitutional clinical trials group supported by the U.S. National Cancer Institute, conducted a study of women with a cytologic diagnosis of AGC. Twenty-five institutions in the U.S. participated in our study (identified in the Acknowledgements). The objective of our study, GOG protocol #171, was to determine whether CA-IX expression in a conventional Pap smear is a diagnostic biomarker for a significant cervical lesion in women with a cytologic diagnosis of AGC, and to explore the diagnostic value of HPV testing alone or in combination with CA-IX.

Material and methods

GOG protocol #171 was initiated in 1998 when the criteria of AGC diagnosis for patient enrollment was based on the 1991 TBS classification and the conventional study Pap smears were used. HPV testing in a liquid-based cytology specimen was added as a study amendment in 2003, and the protocol was closed for accrual in 2005.

Patients

Women over the age of 18 years, with a referring diagnosis of AGC who were expected, on a clinical basis, to undergo complete histologic evaluation of the cervical transformation zone were enrolled. Patients with a history of endometrial hyperplasia and/or carcinoma of the uterine corpus, cervix and vagina; prior or concurrent chemotherapy and/or radiation to the uterine corpus, cervix and vagina; or HIV infection were excluded. Informed consent consistent with federal, state and local requirements was obtained before enrollment. Before activation, the protocol was approved by the National Cancer Institute, Division of Cancer Prevention, and the GOG Human Research Committee, and annually by the Institutional Review Board at each of the participating institutions.

Primary end point and clinical management

The primary end point of the study was complete histologic evaluation of the cervical transformation zone within 6 months of the initial cytologic diagnosis. H&E-stained slides of the most abnormal lesions from each diagnostic procedure were reviewed centrally by teams of 2 pathologists from the GOG Pathology Committee, who reached a consensus diagnosis. Disparities were arbitrated by a 3rd GOG pathologist. Evaluation of the entire transformation zone was required to make a negative diagnosis but not to make a positive diagnosis. A positive diagnosis, coded as a significant cervical lesion (SCL), reflects the presence of CIN2, CIN3, AIS or invasive carcinoma. A negative diagnosis represents the absence of SCLs and includes CIN1 and atypia. Atypia is defined as glandular and squamous lesions in which cellular atypia falls short of AIS and CIN1. The significant lesions were restricted to the cervix, and there was no case of vaginal dysplasia/neoplasia without the coexisting cervical lesions identified in the study. Cases with neoplasia detected in tissues outside of the cervix, where the cervix was histologically confirmed as benign, were classified as negative.

Patients received colposcopic examination, cervical biopsy, endocervical curettage and/or an endometrial biopsy as clinically indicated, as well as LEEP cone biopsy of the cervix with an endocervical curettage, a cold knife cone biopsy of the cervix with/without an endocervical curettage or a hysterectomy within 6 months of the initial cytologic diagnosis of AGC. An endometrial biopsy or curettage was obtained in all perimenopausal and postmenopausal women, as well as in all patients with a negative cone biopsy of the cervix. Patients with a negative diagnosis after the cervical cone biopsy but not undergoing a hysterectomy were to be followed by the relevant referring gynecologist or family physician during regular visits with routine Pap smear screening every 6 months for 2 years.

Pap smear and liquid-based cytology specimens

A spray-fixed conventional study Pap smear was prepared before collecting a LBC (ThinPrep, Cytyc/Hologic, Marlborough, MA) specimen. Both specimens were collected using a spatula and cytobrush and were collected before surgical procedures were performed. Each Pap smear collected from all patients enrolled in the study was tested for CA-IX protein expression. All cases of immunostain were manually performed in Dr. Stanbridge's Laboratory at the University of California, Irvine. The presence of high-risk HPV (H-HPV) DNA in a LBC specimen, collected from 2003, was detected using the Digene Hybrid Capture II (HC2) system (Digene Corp., Gaithersburg, MD) at the University of Oklahoma Health Sciences Center.

Detection of CA-IX in a conventional study Pap smear

CA-IX testing was performed in conventional study Pap smears using the anti-CA-IX mouse monoclonal antibody, M75, as described previously.[6,15,20] Specific immunohistochemical staining was defined by the presence of a brown reaction product on the plasma membrane under 40× magnification. Faint staining of the cytoplasm was considered negative. Cytologic criteria for atypical cells, delineated in the Bethesda System classification, were used in the diagnostic classification.[27] Immunostaining was scored as positive (patterns A and B) and negative (patterns C and D) based on the staining intensity (strong *vs.* weak/negative) and immunoreactive patterns (diffuse *vs.* focal). Strong positivity was defined as when the dark brown immunoreactivity was easily identified at a low power magnification (4× or 10×). The diffuse staining pattern was defined as when more than 50% of the atypical cells or normal endocervical cells in the smear exhibited immunoreactivity to CA-IX. The patterns A, B, C and D were defined as: (A) when individual atypical cells and/or cell clusters exhibited specific immunoreactivity, which was either diffuse or focal; (B) when normal looking endocervical cells exhibited focal or diffuse strong specific positive staining; (C) when the normal endocervical cells exhibited focal but weak staining and (D) when there was nonspecific faint cytoplasmic positivity or lack of staining observed at 40× magnification (Fig. 1). A set of teaching smears with samples of known negative and positive CA-IX immunoreactivity was provided by one of the authors (SYL). After the training session, the CA-IX-immunostained smears were evaluated, and the interpretation in each case was recorded independently by 3 cyto/gynecologic pathologists (SYL, WHR and TAB) without the knowledge of histologic diagnosis. Any case with a different interpretation of immunoreactive patterns (A, B, C and D) was considered to be a discrepant case. All discrepant cases were reviewed simultaneously by 3 study pathologists, using a multiheaded microscope. A consensus was obtained when at least 2 of the 3 study pathologists reached agreement. The results of the consensus were recorded as the final score for each patient in the study.

Detection of high-risk HPV DNA in liquid-based cytology specimens

Each LBC specimen was prepared in 20 ml of the PreservCyt Solution (Cytyc/Hologic, Marlborough, MA), and the presence of

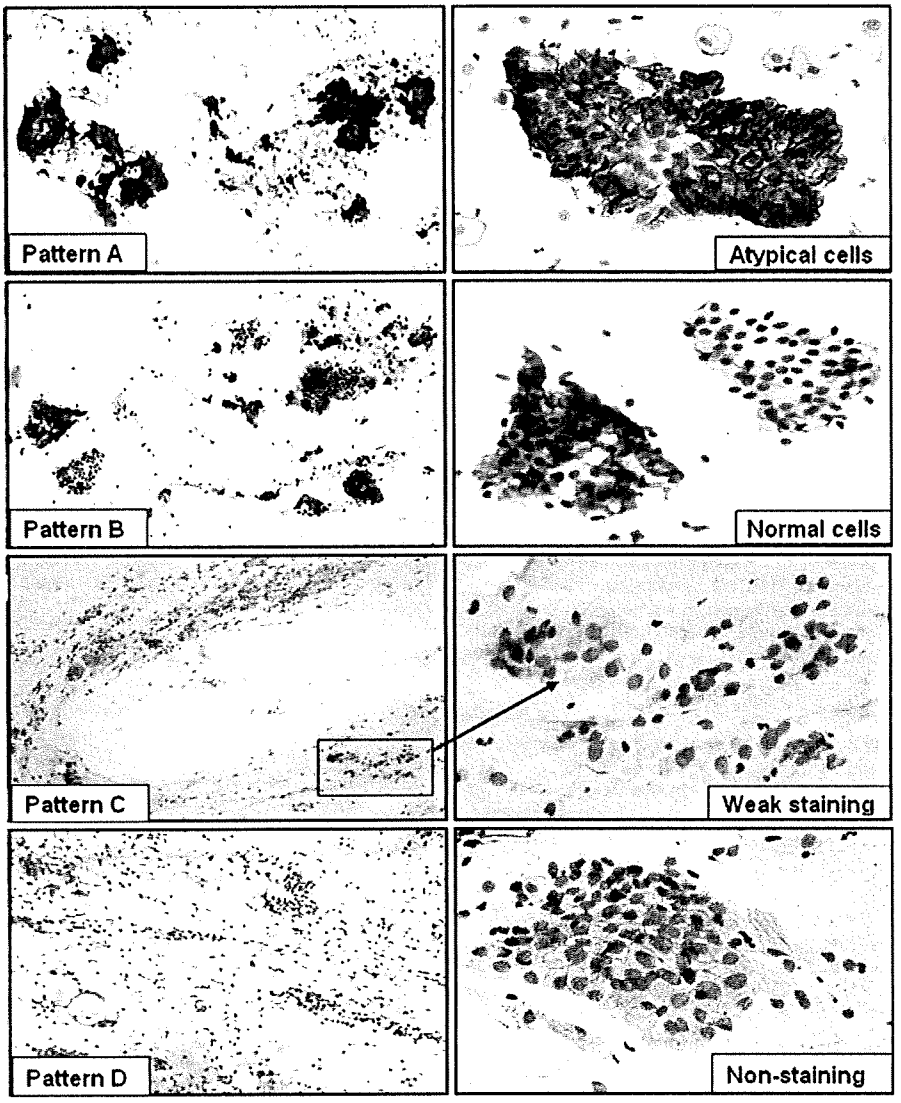

FIGURE 1 – The 4 scoring patterns of CA-IX immunoreactivity in Pap smears containing AGC. Patterns A and B: positive immunostaining in the atypical cells/cell clusters (A) or in the normal looking endocervical cells (B). Patterns C and D: weak positive (C, arrow) or no immunoreactivity (D) in normal cervical cells (original magnification on left panels ×100 and right panels ×400).

H-HPV DNA in at least 4 ml of the LBC specimen was evaluated. The specimens were tested for the presence or absence of H-HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 with the Digene HC2 system, according to the manufacturer's protocol. This test does not distinguish between the different H-HPV types.

PCR-based HPV genotyping

The majority of specimens tested for the presence or absence of H-HPV with the HC2 method were also genotyped using the Roche linear array genotyping test according to the manufacturer's directions. This test uses a combination of amplification of target DNA by the polymerase chain reaction (PCR) and nucleic acid hybridization and has been designed to detect a total of 37 anogenital HPV DNA genotypes, including the same 13 H-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) as the HC2 method. In this case, the method identifies the individual HPV type(s) present in each positive sample.[28,29]

Statistical methods

Statistical analyses were performed using Statistical Analysis System (SAS) version 9.1 (SAS Institute, Cary NC). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), interpreted as the risk of a SCL among women who test negative for HPV and/or CA-IX, and overall accuracy were evaluated using the definition of false-negative rate (FNR) as 1 − NPV to reflect the proportion of negative diagnoses that were incorrect for women diagnosed by CA-IX or HPV status, individually or jointly, relative to histologic diagnosis. When used in combination, the following decision rule was used: diagnose those women with positive CA-IX or positive HPV as having a SCL.

Results

GOG protocol #171 was initiated in 1998 when the criteria of AGC diagnosis for patient enrollment was based on the 1991 TBS

TABLE I - CLINICAL CHARACTERISTICS AND HISTOLOGY DIAGNOSIS

Patient age				Race and ethnicity n = 403 (%)[1]			
Age	n = 403 (%)[1]	n = 122 (%)[2] HPV (HC2)		Race		Ethnicity	
		HPV (+)	HPV (−)	White	268 (67)	Hispanic	99 (25)
≤30	64 (16)	8 (6.5)	8 (6.5)	African American	48 (12)		
31–40	102 (25)	23 (19)	13 (11)	Asian	8 (2)	Non-Hispanic	287 (71)
41–50	115 (29)	9 (7)	31 (25)	American Indian	1		
51–60	72 (18)	7 (6)	15 (12)	Not specified	78 (19)	Not specified	17 (4)
61–70	37 (9)	1 (1)	6 (5)				
≥71	13 (3)	0	1 (1)				

Histologic diagnosis n = 403 (%)[1]					
Insignificant cervical lesions 292 (72)		Significant cervical lesions 111 (28)			
Negative/benign	232	Squamous lesions	74 (67)	Glandular lesions	37 (33)
Negative, NOS	156	CIN2	14	AdenoCa. *in situ*	23
Metaplasia, NOS	19			Invasive AdenoCa.	14
Squamous metaplasia	53	CIN3	58	AdenoCa. NOS	7
Immature squamous Metaplasia	4	Squamous cell Ca.	2	Endometrioid	1
Atypia[3]	23			Clear cell	1
Glandular hyperplasia	5			Small cell	1
CIN1/mild dysplasia	32			Adenosquamous	2
				Villoglandular	1
				Serous	1

CIN2, moderate dysplasia; CIN3, severe dysplasia/*in situ* squamous cell carcinoma; NOS, not otherwise specified.
[1]Four hundred and three cases were tested for CA-IX expression.–[2]Among 403 cases, 122 were tested for high-risk HPV.–[3]Including glandular and squamous lesions in which cellular atypia falls short of AIS and CIN1.

classification and the conventional study Pap smears were used. HPV testing in a liquid-based cytology specimen was added as a study amendment in 2003 to incorporate advances in scientific understanding and the clinical management of women with cervical lesions. The protocol was closed for accrual in 2005.

Between September 28, 1998 and October 10, 2005, 592 women with a cytologic diagnosis of AGC were enrolled in the study. One hundred eighty-nine women were excluded from the study. The reasons for exclusions were as follows: withdrew consent (n = 3), lack of histological evaluation of the transformation zone (n = 110) and unsatisfactory study Pap smears (n = 76). The clinical characteristics and histologic diagnosis of the 403 evaluable women are presented in Table I. The ages ranged from 20 to 86 with a median of 43 years old. The distribution by race and ethnicity is illustrated in Table I.

Table I also provides the distribution of benign and neoplastic cervical lesions observed in this cohort. Of 403 patients enrolled in the study, 111 (28%) women had a SCL, 74 (18%) had significant squamous lesions (SLs) and 37 (9%) had significant GLs. Among those with a SCL (n = 111), 67% were SLs, including CIN2 (n = 14), CIN3 (n = 58) and squamous cell carcinoma (n = 2), and 33% were GLs, including AIS (n = 23) and invasive adenocarcinoma (n = 14). The incidence of invasive carcinoma in the study was 4%. There were 5 women whose malignancy was found outside of the cervix. These malignant tumors involved the endometrium (n = 3), ovary (n = 1) and fallopian tube (n = 1). All of these women with extracervical malignancy were diagnosed during the evaluation of AGC, either by endometrial biopsy or by abnormal physical and radiographic abnormalities. All 5 women received a total hysterectomy and bilateral salpingo-oophorectomy. The cervix of each of these women was histologically confirmed to lack evidence of a significant lesion. The study was designed to focus only on significant lesions of the cervix; thus, these cases were coded as negative for the purposes of the CA-IX and HPV analyses

Figure 1 illustrates the 4 CA-IX immunohistochemical staining patterns. Diffuse or focal CA-IX immunoreactivity in the atypical cells (pattern A) and focal strong or diffuse immunoreactivity in normal looking endocervical cells (pattern B) were classified as positive. CA-IX immunoreactivity that was focal and weak in normal endocervical cells (pattern C) or negative (pattern D) was classified as negative. Pattern A was easily discriminated from patterns C and D. The rate of agreement among any 2 of the 3 reviewers reached 97% on pattern A and 100% on pattern D before the arbitrating consensus review. Five cases were upgraded from pattern C to pattern A, and 5 cases from pattern C to pattern B, but only 2 cases were downgraded from pattern B to pattern C after the consensus review.

CA-IX testing

The intent of the initial study was to determine the accuracy of CA-IX expression exclusively as an indicator of the presence of a SCL. Thus, the data shown in the upper panel (#1) of Table II depict the results of CA-IX testing for all of the specimens (n = 403). Positive staining for CA-IX protein expression was observed in 118 (29%) conventional study Pap smear specimens. Among these positive cases, 83 (70%) had a SCL, including 48 of 74 (65%) SLs and 35 of 37 (95%) GLs. Among 35 insignificant cervical lesions with positive CA-IX immunoreactivity, 2 were CIN1, 1 was atypia and 1 was glandular hyperplasia. Thus, positive CA-IX immunoreactivity in a conventional Pap smear had an overall sensitivity and specificity of 75 and 88%, respectively, with a FNR of 10%, for detecting a SCL. The sensitivity for CA-IX detection in GLs was 95% and in SLs was 65%. (Table III).

A direct comparison between CA-IX expression and HPV detection was performed on those specimens where a LBC specimen was available for HPV analysis (n = 122). For these cases, 31 (25%) were positive for CA-IX expression. Among these CA-IX-positive cases, 25 (80%) had a SCL, including 16 of 28 (57%) SLs and 9 of 10 (90%) GLs. Thus, the overall sensitivity of SCLs, SLs or GLs was 66, 57 or 90%, respectively, with a specificity of 93% and a FNR of 14%. The comparative analysis of CA-IX and HPV is given later and in Tables II and III.

HPV detection

The HC2 method of HPV testing was performed on 122 cases. Patient ages ranged from 20 to 71, with a median age of 36 for the HPV-positive group and 45 for the HPV-negative group (Table I). H-HPV DNA was detected in 48 (39%) of LBC specimens (Table II, #2). Among these positive cases, 37 (77%) had a SCL, includ-

TABLE II – BIOMARKER TEST (CA-IX, HPV AND CA-IX + HPV) RESULT BY HISTOLOGIC DIAGNOSIS

Biomarker test		Histologic diagnosis			
		Insignificant cervical lesions	Squamous lesions (SLs)	Glandular lesions (GLs)	All SCLs (SLs + GLs)
Total number (#1)	403 (%)	292 (73)	74 (18)	37 (9)	111 (27)
CA-IX					
Negative	285 (71)	257 (88)	26 (35)	2 (5)	28 (25)
Positive	118 (29)	35 (12)[1]	48 (65)	35 (95)	83 (75)
Total number (#2)	122 (%)	84 (69)	28 (23)	10 (8)	38 (31)
CA-IX					
Negative	91 (75)	78 (93)	12 (43)	1 (10)	13 (34)
Positive	31 (25)	6 (7)	16 (57)	9 (90)	25 (66)
HPV (HC2)					
Negative	74 (61)	73 (87)	1 (4)	0	1 (3)
Positive	48 (39)	11 (13)[2]	27 (96)	10 (100)	37 (97)
CA-IX + HPV					
Negative	68 (56)	67 (80)	1 (4)	0	1 (3)
Positive	54 (44)	17 (20)	27 (96)	10 (100)	37 (97)

#1: All cases tested for CA-IX; #2: Cases in which HPV (HC2) results were available.
Insignificant cervical lesions including negative/benign, CIN1, atypia and glandular hyperplasia; SCLs: significant cervical lesions; SLs: CIN2, CIN3 and squamous cell carcinoma; GLs: AIS and adenocarcinoma.
[1]Including 2 CIN1, 1 atypia and 1 hyperplasia.–[2]Including 4 CIN1 and 1 atypia.

TABLE III – DIAGNOSTIC ACCURACY OF CA-IX, HPV AND CA-IX + HPV

Numbers tested	Biomarkers	Sensitivity			Specificity	Negative predictive value (NPV)	Positive predictive value	False-negative rate (1 – NPV)[1]
		Significant cervical lesions	Squamous lesions	Glandular lesions				
403	CA-IX	0.75	0.65	0.95	0.88	0.90	0.70	0.10
122								
	CA-IX	0.66	0.57	0.90	0.93	0.86	0.81	0.14
	HPV (HC2)	0.97	0.96	1.00	0.87	0.99	0.77	0.01
	HPV (HC2) + CA-IX	0.97	0.96	1.00	0.80	0.99	0.69	0.01

[1]FNR defined as 1 – NPV to reflect the proportion of negative diagnoses that were incorrect.

ing 27 of 28 (96%) SLs and 10 of 10 (100%) GLs. This provided an overall sensitivity of 97, 96 and 100% for SCLs, SLs and GLs, respectively, with a specificity of 87% and a FNR of 1% (Table III). There were 11 cases in the insignificant lesion category, and among these, 4 were diagnosed as CIN1 and 1 was atypia.

HPV genotyping

One hundred twelve of 122 cases tested for the presence of HPV by the HC2 method were also processed for HPV genotyping, using the PCR-based Roche linear array (RLA) kit. Sixty-five cases (58%) were positive for H-HPV. Among these positive cases, 36 (54%) had SCLs, including 26 of 27 (96%) SLs and 10 of 10 (100%) GLs. Thus, the PCR-based HPV genotyping method for detecting SCLs gives an overall sensitivity and specificity of 97 and 61%, respectively, and a FNR of 2%. In terms of the rates of positive HPV detection, the PCR-based RLA and HC2 methods did show some differences, with the RLA method detecting more positives (39 *vs.* 13%) in the insignificant lesion category and less (97 *vs.* 100%) in the SCL category, respectively. The comparative analysis of HC2 testing and PCR-based RLA genotyping is given in Table IV.

A compilation of the genotyping data revealed that ~49% of both benign lesions and SCLs contained multiple HPV types (Table V). There was no apparent difference with respect to whether the lesion was positive or negative by the HC2 method of detection (data not shown). The distribution of the specific H-HPV types in the cervical lesions is shown in Table VI.

The combined accuracy of CA-IX and HPV testing

The data were also evaluated based on the combined CA-IX and HPV testing (HC2 method) performed in 122 cases. For the combination of CA-IX with HPV, a case was negative if there was a negative result for both CA-IX and HPV. If either or both were positive, the case was then called positive. The combined CA-IX and HPV testing had an overall sensitivity and specificity of 97 and 80% and a FNR of 1%. Details are given in Table III.

Discussion

GOG protocol #171 is the 1st cooperative group-wide prospective cohort study of women with AGC diagnoses. The participants were enrolled from 25 medical institutions. In agreement with previous studies, a wide spectrum of benign and clinical significant lesions was identified in patients with a diagnosis of AGC enrolled in the study.[6–9] The rate of significant uterine lesions in published studies of AGC has ranged from 17 to 80% (mean, 41%), with ranges of 0–34% (mean, 11%) with GLs, 5–43% (mean, 17%) with SLs and 0–23% (mean, 9%) with invasive carcinomas. Most of this latter category were of endocervical or endometrial origin.[9] In the study described here, we found 28% of women had a SCL (CIN2, CIN3, AIS or invasive carcinoma), 18% had a SL and 9% had a GL. The overall rate of invasive carcinoma was 4%. Among the SCLs, 33% were GLs (AIS/adenocarcinomas) and 14% were invasive cervical carcinomas. These results are similar to the published findings.[6–9]

With the exception of 2 adenocarcinomas, all AIS and conventional endocervical adenocarcinomas exhibited the strong and diffuse CA-IX immunoreactive pattern A, which is easily discriminated from the weak or negative immunostaining patterns under 4× or 10× magnification. The observations of diffuse positivity in cases of AIS and adenocarcinoma are identical to those previously reported; thus, our study confirms the diagnostic utility of CA-IX for glandular neoplasia of the cervix.[15] Although CA-IX testing missed 2 adenocarcinomas, no abnormal cells were seen in the Pap smear (sampling error) in 1 case, and the other was a clear

TABLE IV – THE COMPARISON OF HYBRID CAPTURE 2 (HC2) AND PCR-BASED ROCHE LINEAR ARRAY (RLA) HPV DETECTION

HPV Test		Histologic diagnosis				Sensitivity			Specificity	Positive predictive value	False-negative rate
		Insignificant cervical lesions	Squamous lesions (SLs)	Glandular lesions (GLs)	All SCLs (SLs + GLs)	Significant cervical lesions	Squamous lesions (SLs)	Glandular lesions (GLs)			
HC2											
Total Number	122 (%)	84 (69)	28 (23)	10 (8)	38 (31)	0.97	0.96	1.00	0.87	0.81	0.01
Negative	74 (61)	73 (87)	1 (4)	0	1 (3)						
Positive	48 (39)	11 (13)	27 (96)	10 (100)	37 (97)						
PCR-RLA											
Total Number	112 (%)	75 (67)	27 (24)	10 (9)	37 (33)	0.97	0.96	1.00	0.61	0.55	0.02
Negative	47 (42)	46 (61)	1 (4)	0	1 (3)						
Positive	65 (58)	29 (39)	26 (96)	10 (100)	36 (97)						

Insignificant cervical lesions including negative/benign, CIN 1, atypia and glandular hyperplasia; SCLs: significant cervical lesions; SLs: CIN2, CIN3 and squamous cell carcinoma; GLs: AIS and adenocarcinoma.

TABLE V – DETECTION OF HIGH-RISK HPV TYPES BY PCR-BASED ROCHE LINEAR ARRAY TESTING

Histologic diagnosis	Total number of HPV	Multiple HPV types	Single HPV type
	Positive cases *n* (%)	(With or without low risk)	
Total numbers (Negative + positive lesions)	65 (%)	32 (49)	33 (51)
Negative/benign	29 (45)	13 (45)	16 (55)
CIN2, CIN3 (moderate/severe dysplasia/ *in situ* squamous carcinoma)	25 (38)	12 (48)	13 (52)
Squamous cell carcinoma	1 (1)	0	1 (100)
Adenocarcinoma *in situ*	5 (8)	4 (80)	1 (20)
Invasive adenocarcinoma	5 (8)	3 (60)	2 (40)

TABLE VI – HIGH-RISK HPV GENOTYPES USING THE ROCHE LINEAR ARRAY KIT

Histologic Diagnosis/HPV type	16	18	31	33	35	39	45	51	52	53	56	58	59	66	68	82
Negative/benign	4	2	4	1	1	1		1		2		1				
Glandular hyperplasia																
Atypia	1	1														
CIN1 (mild dysplasia)		1	1	1		3		2	1	2	2	2	2	1		
CIN2 (moderate dysplasia)	1		1	1	2			1	1							
CIN3 (severe dysplasia/*in situ* squamous Ca.	8	1	4		2	1	3	1	1	1		4	2			
Squamous cell Ca.		1														
Adenocarcinoma *in situ*	1	3					1									
Adenocarcinoma, NOS	2	1								1				1	1	
Adenosquamous cell Ca.		1														
Villoglandular AdenoCa.							1						1			
Total number	17	11	10	3	5	5	5	5	3	6	2	8	5	2	1	0

cell adenocarcinoma of the cervix, which does not express CA-IX.[15] There were 5 cases in which malignancy was identified outside of the cervix, including endometrium (3), fallopian tube (1) and ovary (1), but in all of these cases the cervix was histologically confirmed to be benign. In each instance, there were few exfoliated atypical cells in the smears, and all 3 carcinomas of the endometrium were diagnosed by endometrial biopsy. In the 2001 TBS, these atypical cells would be classified as atypical endometrial cells. Furthermore, the goal of the cervical Pap screening program does not include identification of malignancies originating outside of the uterus.

H-HPV DNA has been strongly associated with SCLs; however, only limited, albeit promising, published data on HPV testing with AGC exist. In our study, we detected H-HPV types in 39% of the 122 women enrolled, including 96% of those with CIN2, CIN3 or squamous cell carcinoma, and 100% with AIS and adenocarcinoma. Of the 122 cases tested by HC2, 112 were genotyped using the PCR-based RLA genotyping kit. The sensitivity of detection of H-HPV in SCLs is similar between the 2 methods, and this also has been seen in other large population studies.[28,30,31] However, significantly more cases with benign lesions were positive by the RLA method. Another interesting feature of the H-HPV analysis is that, in the positive cases, multiple HPV types were detected in both benign and SCLs at approximately equal frequencies to those that identified individual genotypes.

Our data suggest that, in the U.S., H-HPV testing is a useful biomarker for identifying SCLs in women with a cytologic diagnosis of AGC, and has a sensitivity of 97% and specificity of 87%, with a false negative rate of 1%. CA-IX expression is less sensitive than HPV in detecting SLs (65 *vs.* 96%, respectively). The sensitivity of HPV for detection of GLs appears to be also better than CA-IX (100% [10/10] *vs.* 95% [35/37]). However, the confidence intervals overlapped and the difference did not achieve statistical significance. This trial was not designed to detect such a difference.

A replacement protocol (GOG-237) has been activated, using LBC specimens, to evaluate the diagnostic accuracy of H-HPV DNA in combination with CA-IX, p16, minichromosome maintenance proteins and/or Ki67, for cervical dysplasia/neoplasia in women with a cytologic diagnosis of AGC. In the future study, all experimental procedures, including HPV testing protocols, will be identical. The major goal of the future research will be to determine whether the specificity and PPV of HPV testing can be improved by incorporating any of these additional biomarkers into the diagnostic test, without sacrificing the excellent sensitivity observed in our study for H-HPV alone.

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REVIEW ARTICLE

Improving Cancer Outcomes Through International Collaboration in Academic Cancer Treatment Trials

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ABSTRACT

Purpose
The need for international collaboration in cancer clinical trials has grown stronger as we have made progress both in cancer treatment and screening. We sought to identify those efforts already underway which facilitate such collaboration, as well as barriers to greater collaboration.

Methods
We reviewed the collective experiences of many cooperative groups, governmental organizations, nongovernmental organizations, and academic investigators in their work to build international collaboration in cancer clinical trials across multiple disease sites.

Results
More than a decade of work has led to effective global harmonization for many of the elements critical to cancer clinical trials. Many barriers remain, but effective international collaboration in academic cancer treatment trials should become the norm, rather than the exception.

Conclusion
Our ability to strengthen international collaborations will result in maximization of our resources and patients, permitting us to change practice by establishing more effective therapeutic strategies. Regulatory, logistical, and financial hurdles, however, often hamper the conduct of joint trials. We must work together as a global community to overcome these barriers so that we may continue to improve cancer treatment for patients around the world.

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INTRODUCTION

As improvements in cancer treatment have led to increased survival, the need for expanded collaboration on treatment trials has correspondingly increased. First, new active treatments which prolong survival in turn often require larger sample sizes to detect potential benefit from experimental regimens or to determine the similar efficacy of a less toxic regimen. Second, developments in molecular biology have allowed the use of molecular markers to define patient cohorts based on tumor biology.[1] As a result, we must cast a wider net to enroll the necessary number of patients with the appropriate molecular classification within a reasonable timeframe. Third, the success of effective screening and earlier diagnosis has decreased the incidence of advanced-stage disease for certain cancers in developed countries. Fourth, targeted therapy may offer effective treatment for relatively rare tumor types or rare subtypes of common cancers. Fifth, the integration of the plethora of new cancer treatment agents into existing treatment regimens will require the rapid conduct of phase III trials so that results are relevant to current clinical practice. Finally, the completion of larger trials across multiple countries will assure the broad applicability of research findings worldwide as well as facilitate the uptake of improvements in cancer treatment into standard practice. The following discussion focuses on efforts made to facilitate global collaboration, as well as some of the barriers to such collaboration. Both clinical investigators and policy makers need to be aware of these issues.

FACILITATING INTERNATIONAL COLLABORATION IN CLINICAL TRIALS

Exchange of Information on Clinical Trials

The importance of a central registry for clinical trials was underscored by the WHO with the

creation of the WHO International Clinical Trials Registry Platform in 2005.[2] Registries contributing to this global trials registry include the US National Library of Medicine registry, called Clinical Trials.gov, and the National Cancer Institute (NCI) registry of United States and international cancer clinical trials.[3,4] The European Commission is considering the establishment of a public database for all clinical trials conducted in the European Union.

Harmonization of Staging, Classification, and End Points Definition

Consensus on standards for disease classification, staging, and trial end points is required to make international collaboration in clinical trials successful. At present, the International Union Against Cancer works in conjunction with the American Joint Committee on Cancer and the International Federation of Obstetrics and Gynecology to maintain and update the current cancer staging system.[5] The WHO and the International Agency for Research on Cancer have led efforts toward standardization of pathologic diagnoses through publication of the International Classification of Diseases for Oncology, as well as various monographs on specific cancer sites.[6] More recently, efforts to harmonize molecular staging of cancer have been led by the hematologic oncology and pediatric oncology communities.[1,7-12]

Regulatory authorities in Europe, Japan, and the United States established the International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use in 1990.[13] The International Conference on Harmonisation issued a common technical document in 2000; it continues to work on harmonization for drug development and registration. As part of implementation of these efforts, the US Code of Federal Regulations now makes clear that foreign clinical data can be the sole basis for granting marketing approval to a new drug by the US Food and Drug Administration.[14] Several agents, including bevacizumab for metastatic breast cancer, temozolamide in conjunction with radiation for newly diagnosed gliomas, and letrozole for early, hormone receptor–positive breast cancer in postmenopausal women, were approved for marketing in the United States and Europe based only on data from clinical trials conducted outside those jurisdictions.[15-17]

The NCI, the National Cancer Institute of Canada Clinical Trials Group (NCIC-CTG), and the European Organisation for Research and Treatment of Cancer (EORTC) have undertaken to harmonize adverse event reporting and data capture for cancer clinical trials. These harmonization efforts have included the development of common nomenclature and scoring for treatment-related toxicity and adverse events.[18-20]

Objective assessment of tumor response in both solid and hematologic tumors has recently been the subject of several international efforts. The Response Evaluation Criteria in Solid Tumors Working Group (with membership from NCI, EORTC, NCIC-CTG, supplemented with input from the nine NCI-sponsored clinical trials groups, pharmaceutical industry, and regulators) published criteria for response assessment in 2000, which was updated in 2008.[21,22] These criteria have been endorsed by regulatory bodies such as the US Food and Drug Administration and the European Medicines Agency. The NCI and EORTC are currently revisiting the 1999 recommendations regarding use of [18F]-fluorordexoyglucose and positron emission tomography for use in evaluating tumor response.[23] Similarly, recommendations for standard response criteria for lymphomas and acute myeloid leukemia have been published.[24,25]

The Breast Cancer Intergroup of North America has recognized the need for harmonization of clinical end points in trials of adjuvant treatment for breast cancer. They have proposed "standardized definitions for efficacy end points."[26,27]

Other harmonization efforts have included the development of standard protocol language for surgical procedures, the details of chemotherapy administration, and supportive care measures. The International Atomic Energy Agency is working to develop harmonization for radiation treatment planning and dosing in cancer treatment trials.

Challenges to International Collaboration

In the face of these efforts to increase participation in clinical trials and to facilitate international collaboration, national and regional regulatory authorities have heightened the level of oversight and regulation required for clinical trials in recent years. Therefore, when trials are conducted in multiple jurisdictions, an increasingly complex array of differing regulations apply. For example, in 2001, the European Union issued a directive concerning clinical trials of medicinal products to ensure compliance with the International Conference on Harmonisation Good Clinical Practice guidance.[28] This directive affects the conduct of almost all phase I, II, and III trials assessing a drug or drugs. The requirements of the European Union Clinical Trials Directive, which were developed for industry-sponsored studies, have hampered the opening of clinical studies with academic sponsors that often do not have the resources available to meet the expanded regulatory obligations.[29] In addition, the European Union Clinical Trials Directive has also slowed collaboration between European investigators and those outside the European Union. Implementation of this directive has varied from country to country within the European Union, adding to the level of complexity and staff requirements.[30]

The US Department of Health and Human Services Office of Human Research Protection has mandated that all research sites outside of the United States that participate in research funded by the US government must file documentation certifying that each research site observes the Declaration of Helsinki on Ethical Principles for the Conduct of Research on Human Subjects and has an independent ethics committee.[31] Sites participating in trials sponsored by the NCI must also undergo regular on-site audits.[32]

Both the systems and forms for reporting study-related adverse events can vary from country to country, although most cooperative groups and academic institutions do use the harmonized criteria for categorizing and grading adverse events. In addition, companies often differ in their requirements for reporting adverse events, as well as their interpretation of each country's regulatory requirements.

INDEMNITY INSURANCE

In many countries, independent ethics committees/institutional review boards may require indemnity or clinical trial insurance for institutions for non-negligent harm resulting from clinical research, as well as insurance coverage for patients for untoward events. In the European Union, such insurance is required by the European Union Clinical Trials Directive. Insurance availability often varies by country.

TUMOR AND SPECIMEN COLLECTION

The growing interest in establishing the molecular determinants of outcome and of predictors of therapeutic benefit has led to the frequent incorporation of translational biologic questions in randomized trials. Both exploratory and validation studies may have implications for intellectual property issues relating to correlative biology.

To address these translational questions, collection of tumor and other specimens from each patient enrolled is thus becoming increasingly commonplace. Shipment of specimens across international borders may require permission from a national oversight body or may be forbidden altogether. In some cases, it may be necessary to set up parallel specimen banks and core laboratories in each country or region. If multiple specimen banks and core laboratories are established, however, the trial will need to institute quality assurance procedures to ensure that all specimen banking and analyses are performed using the same techniques.

As correlative science techniques have evolved, so has the need for harmonization of tissue collection, processing, and testing. The NCI has recently published guidelines for tissue acquisition, as has the EORTC.[33] The North American cooperative groups and the Breast International Group have formulated breast cancer–specific guidelines, which they have agreed to incorporate in future studies.[34]

IMAGING FOR STAGING, TREATMENT PLANNING, AND EVALUATION OF RESPONSE

As cancer imaging has grown more sophisticated, the need for quality assurance and quality control of imaging studies has also grown. Therefore, international collaboration in cancer clinical trials often requires the development of guidelines for imaging studies, plans for routine central review of some or all studies, and consideration of a virtual imaging bank in which digitized imaging studies from patients on clinical trials can be collected and reviewed. The NCI, working in collaboration with cooperative groups with expertise in image acquisition, the American College of Radiology Imaging Network, the Quality Assurance Review Center, and the Cancer and Leukemia Group B imaging core laboratory at Ohio State University, developed a virtual imaging evaluation workspace in 2007. The consortium has established an imaging core service and repository with capability of acquiring and storing image objects on a worldwide basis. In addition, the same collaborators plan to develop standard operating procedures for assessment of imaging end points in cancer as well as evaluation of new imaging markers.

RADIATION THERAPY

As a critical modality for cancer treatment, radiation in clinical trials must undergo similar processes for quality assurance and quality control as other modalities of treatment. The NCI supports quality assurance for radiation dosimetry in NCI-sponsored trials through the Radiological Physics Center, quality assurance for radiation delivery methods through the Radiation Therapy Oncology Group and the Quality Assurance Review Center, and, more recently, quality assurance for advanced-technology radiation therapy (eg, three-dimensional conformal radiation therapy, stereotactic radiation therapy, intensity modulation therapy) through the Advanced Technology Consortium.[35-39] These quality assurance activities have been routinely implemented for NCI-sponsored cancer trials in North America, as well as for select academic and pharmaceutical trials in Europe and Japan. Globally, however, quality assurance requirements, such as facility questionnaires, facility credentialing, external reference dosimetry audits, and phantom measurements, vary from group to group, both in content and evaluation criteria. This variation hampers collaboration and makes comparisons and meta-analyses difficult. In addition, both radiotherapy technology and the tools for quality assurance are constantly evolving. Close engagement between clinical trialists and manufacturers is required to integrate new digital formats smoothly and ensure that a common framework for data interpretation can achieve a uniform level of quality.

FINANCIAL AND LOGISTICAL SUPPORT

The ability to conduct cancer clinical trials efficiently requires ongoing support for infrastructure, both centrally and at participating institutions. Building the infrastructure for a specific trial is much less efficient than building and maintaining infrastructure for an ongoing series of trials. The central and institutional costs for cancer treatment trials are summarized in Tables 1 and 2. Support for these costs may come from a variety of sources, including government, industry, charity, and local academic institutional contributions. Government support has varied from country to country and region to region. The NCI began to support the infrastructure for cancer clinical trials in 1956. In 2007 the NCI's budget for the US-based nine clinical trials cooperative groups, which together enroll about 25,000 patients per year to trials, was approximately $145 million. Over the past 10 years, the United Kingdom has formalized and provided centralized funding for standing clinical trials networks throughout the country, initially for oncology, and now for medical research of all types. The United Kingdom provides infrastructure support to all clinical sites participating in approved phase II and III trials and large cohort studies through the National Cancer Research Network. Publicly funded charities such as Cancer Research UK and government agencies, such as the Medical Research Council, provide support for both early- and late-phase

Table 1. Central Costs for Cancer Treatment Trials

Protocol design and development, including support for meetings and conference calls
Preparation of applications to central regulatory authorities and central ethics authorities, as applicable
Collection/monitoring of institutional and investigator regulatory compliance
Verification of patient eligibility and management of treatment assignment
Clinical trial insurance
Patient random assignment
Database development
Data collection and management
Drug supply and distribution
Statistical design and analysis
Tumor, specimen and imaging banking
Quality assurance/quality control
Onsite monitoring and audits of participating sites
Pharmacovigilance