original articles

Two biomarker-directed randomized trials in European and Chinese patients with nonsmall-cell lung cancer: the BRCA1-RAP80 Expression Customization (BREC) studies[†]

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Background: In a Spanish Lung Cancer Group (SLCG) phase II trial, the combination of BRCA1 and receptor-asso-
ciated protein 80 (RAP80) expression was significantly associated with outcome in Caucasian patients with nonsmall-cell
lung cancer (NSCLC). The SLCG therefore undertook an industry-independent collaborative randomized phase III trial
comparing nonselected cisplatin-based chemotherapy with therapy customized according to BRCA1/RAP80 expres-
sion. An analogous randomized phase II trial was carried out in China under the auspices of the SLCG to evaluate the
effect of BRCA1/RAP80 expression in Asian patients.

Patients and methods: Eligibility criteria included stage IIIB–IV NSCLC and sufficient tumor specimen for molecular
analysis. Randomization to the control or experimental arm was 1 : 1 in the SLCG trial and 1 : 3 in the Chinese trial.

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In both trials, patients in the control arm received docetaxel/cisplatin; in the experimental arm, patients with low RAP80 expression received gemcitabine/cisplatin, those with intermediate/high RAP80 expression and low/intermediate BRCA1 expression received docetaxel/cisplatin, and those with intermediate/high RAP80 expression and high BRCA1 expression received docetaxel alone. The primary end point was progression-free survival (PFS).

Results: Two hundred and seventy-nine patients in the SLCG trial and 124 in the Chinese trial were assessable for PFS. PFS in the control and experimental arms in the SLCG trial was 5.49 and 4.38 months, respectively [log rank $P = 0.07$; hazard ratio (HR) 1.28; $P = 0.03$]. In the Chinese trial, PFS was 4.74 and 3.78 months, respectively (log rank $P = 0.82$; HR 0.95; $P = 0.82$).

Conclusion: Accrual was prematurely closed on the SLCG trial due to the absence of clinical benefit in the experimental over the control arm. However, the BREC studies provide proof of concept that an international, nonindustry, biomarker-directed trial is feasible. Thanks to the groundwork laid by these studies, we expect that ongoing further research on alternative biomarkers to elucidate DNA repair mechanisms will help define novel therapeutic approaches.

Trial registration: NCT00617656/GECP-BREC and ChiCTR-TRC-12001860/BREC-CHINA

Key words: biomarkers, BRCA1, clinical trial, customized chemotherapy, non-small-cell lung cancer, RAP80

introduction

Since 1978, cisplatin has been the foundation of chemotherapy for nonsmall-cell lung cancer (NSCLC). However, in unselected patients, response rates are only 15%–30% and median survival is 10–12 months, while the majority of patients suffer severe side-effects with little therapeutic benefit [1]. The reaction of cisplatin with DNA produces DNA interstrand cross-links, which are repaired by excision repair cross-complementation group 1 (ERCC1), indicating a possible role for ERCC1 in biomarker-directed therapy. Nevertheless, neither an early ERCC1-directed randomized trial by our group [2] nor a later study [3] showed a survival benefit for ERCC1-directed treatment over nonselected chemotherapy.

Breast cancer 1, early onset (BRCA1) plays a critical role in homologous recombination DNA repair [4]. Recent findings show that BRCA1-depleted cells are homologous recombination competent but still hypersensitive to interstrand cross-links, suggesting that BRCA1 has an additional upstream role in processing interstrand cross-links before double-strand break repair [5]. Experimental models have demonstrated that BRCA1 induces a 10–1000-fold increase in resistance to platinums but a dramatic sensitivity to paclitaxel, docetaxel and vinorelbine [6–8]. We observed that higher BRCA1 mRNA levels were associated with higher response and longer progression-free survival (PFS) in patients with NSCLC treated with docetaxel/gemcitabine [9]. However, in a Spanish Lung Cancer Group (SLCG) phase II BRCA1 biomarker-directed study (ClinicalTrials.gov NCT00883480), where patients were treated with cisplatin/gemcitabine, cisplatin/docetaxel or single-agent docetaxel, we found no differences in survival according to BRCA1 levels [10].

Receptor-associated protein 80 (RAP80) plays a major role in homologous recombination and is associated with several proteins [11], including BRCA1 [4]. In the SLCG phase II study, survival was influenced by RAP80 mRNA levels, and in patients with low levels of both BRCA1 and RAP80 receiving cisplatin plus gemcitabine, median survival was longer than 26 months [10]. Similar results were later found in a retrospective analysis of patients treated with platinum plus either gemcitabine or pemetrexed, where median survival was not reached in patients with low levels of both BRCA1 and RAP80 [12].

Despite evidence that BRCA1 and RAP80 expression affect chemotherapy outcome in European NSCLC patients [9, 10, 12],

this combinatory effect has not been examined in Chinese NSCLC patients, although one study in Chinese patients found that BRCA1 expression correlated with *in vitro* chemosensitivity to cisplatin and docetaxel in malignant effusions of NSCLC and gastric cancer patients [13]. In a more recent study in Chinese gastric cancer patients, higher BRCA1 expression levels were associated with longer overall survival (OS) to second-line docetaxel ($P = 0.006$), while no correlation between RAP80 expression and survival was observed [14].

Based on our findings in the phase II study [10], in cooperation with the French Lung Cancer Group (FLCG), the SLCG undertook the BRCA1-RAP80 Expression Customization (BREC) study, an independent, nonindustry, randomized phase III biomarker-directed clinical trial in advanced NSCLC comparing nonselected cisplatin-based chemotherapy with therapy customized according to BRCA1 and RAP80 levels (NCT00617656/GECP-BREC). At the same time, in order to examine the potential effect of BRCA1 and RAP80 in Asian patients, an analogous phase II randomized study was carried out in China under the auspices of the SLCG (ChiCTR-TRC-12001860/BREC-CHINA).

patients and methods

study design and participants

The SLCG randomized phase III clinical trial was conducted at 72 sites in Spain, France, Belgium, Luxembourg and Saudi Arabia from February 2008 to March 2013. The Chinese randomized phase II clinical trial was conducted at 14 sites from October 2010 to February 2013. Inclusion criteria are shown in supplementary Material, available at *Annals of Oncology* online, and in the protocols.

Sample size was based on the comparison of a control and an experimental arm for the primary end point of PFS, calculated from the time of randomization to progression or death. Secondary end points were OS, calculated from the time of randomization to death, response rate according to Response Evaluation Criteria in Solid Tumors (RECIST) [15], toxicities according to the National Cancer Institute Common Terminology Criteria for Adverse Events v 3.0, and translational research, including the study of other potential genetic markers of outcome. Since the primary end point was PFS in the control versusexperimental arms, all subcomparisons between arms or within subgroups were considered exploratory.

The protocols were approved by the institutional review board or independent ethics committee at each participating site and complied with the International Ethical Guidelines for Biomedical Research Involving Human
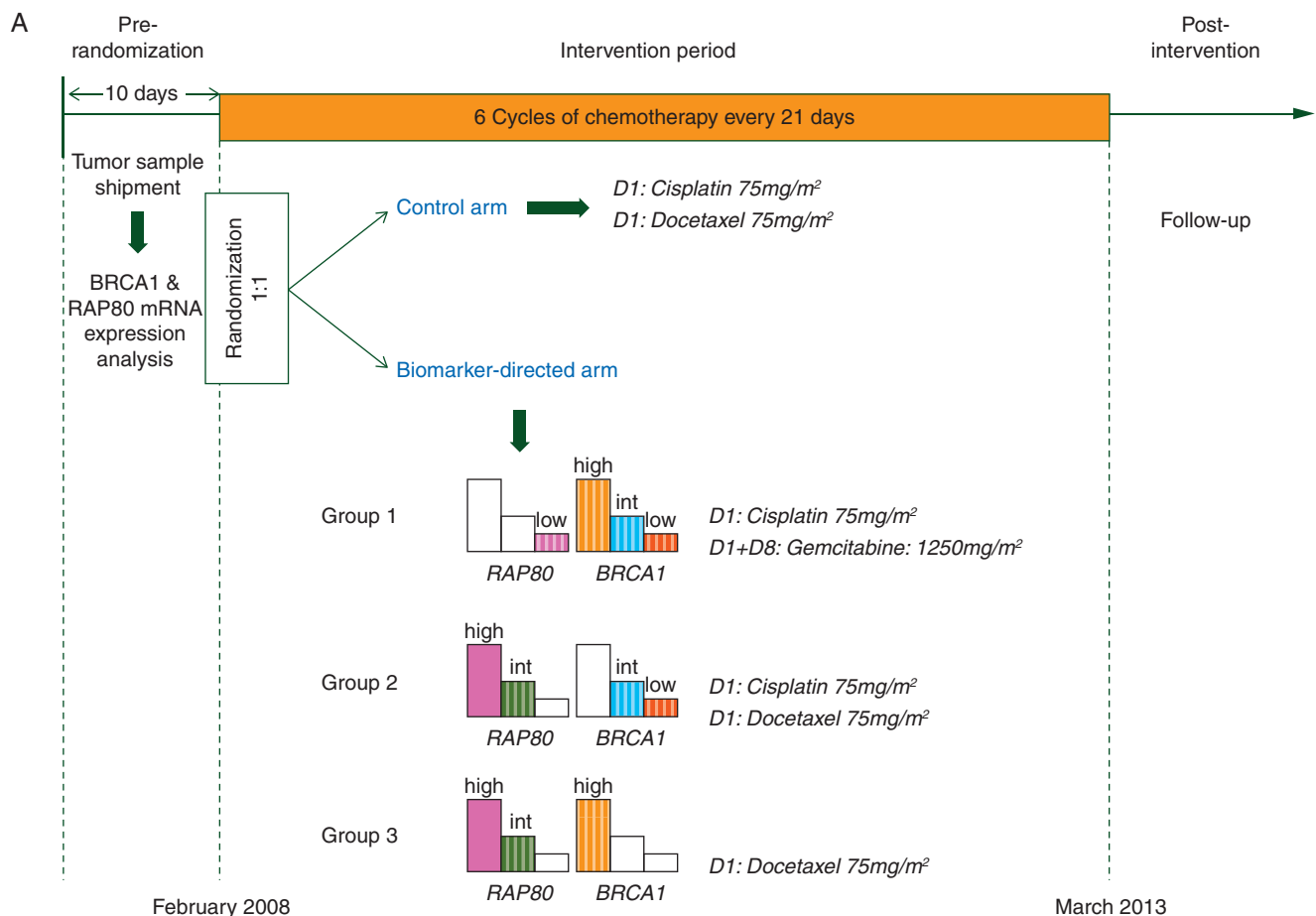
Figure 1. Study design and patient disposition in the SLCG and Chinese studies. (A) During the 10-day initial period, BRCA1 and RAP80 mRNA expression was analyzed, and if the analysis was successful, patients were randomized 1 : 1 in the SLCG study and 1 : 3 in the Chinese study to either the control or bio-marker-directed experimental arm. (B) Patient disposition showing registration, randomization and follow-up of the patients in the SLCG study. Criteria for inclusion in the per-protocol population (assessable patient set) included reception of at least one dose of the study drug and no major protocol violation. Of the 1116 patients who were assessed for eligibility, 734 did not enroll. The main reason for this seemingly high failure rate was an inadequate tumor specimen in 504 patients (45%), which had been foreseen in the protocol. Of 382 randomized patients, 103 were not eligible for inclusion in the per-protocol population. Eighty-seven patients were randomized just before the cutoff for the interim analysis and did not have data available for analysis. An additional eight patients were not included due to inclusion error, and another eight patients did not receive any study treatment. (C) Patient disposition showing registration, randomization and follow-up of the patients in the Chinese study.

Subjects, Good Clinical Practice guidelines, the Declaration of Helsinki and local laws. All patients provided their written informed consent.

In both trials, an initial 10-day period was allocated for the shipment of tumor samples and the analysis of BRCA1 and RAP80. Patients in whom molecular analyses were successful were then randomized for treatment (Figure 1A). Randomization was carried out centrally and automatically by generation of randomized permuted blocks. Patients in both arms were stratified according to histology, Eastern Cooperative Oncology Group (ECOG) performance status (PS), RAP80 expression levels and BRCA1 expression levels.

Patients in the SLCG trial were randomly assigned in a 1 : 1 ratio to either the control or the experimental arm. Patients in the control arm received docetaxel (75 mg/m^2) plus cisplatin (75 mg/m^2) on day 1. In the experimental arm, patients with low RAP80 expression and any level of BRCA1 expression (group 1) received gemcitabine (1250 mg/m^2) on days 1 and 8 plus cisplatin (75 mg/m^2) on day 1; patients with intermediate or high RAP80 expression and low or intermediate BRCA1 expression (group 2) received docetaxel (75 mg/m^2) plus cisplatin (75 mg/m^2) on day 1; patients with
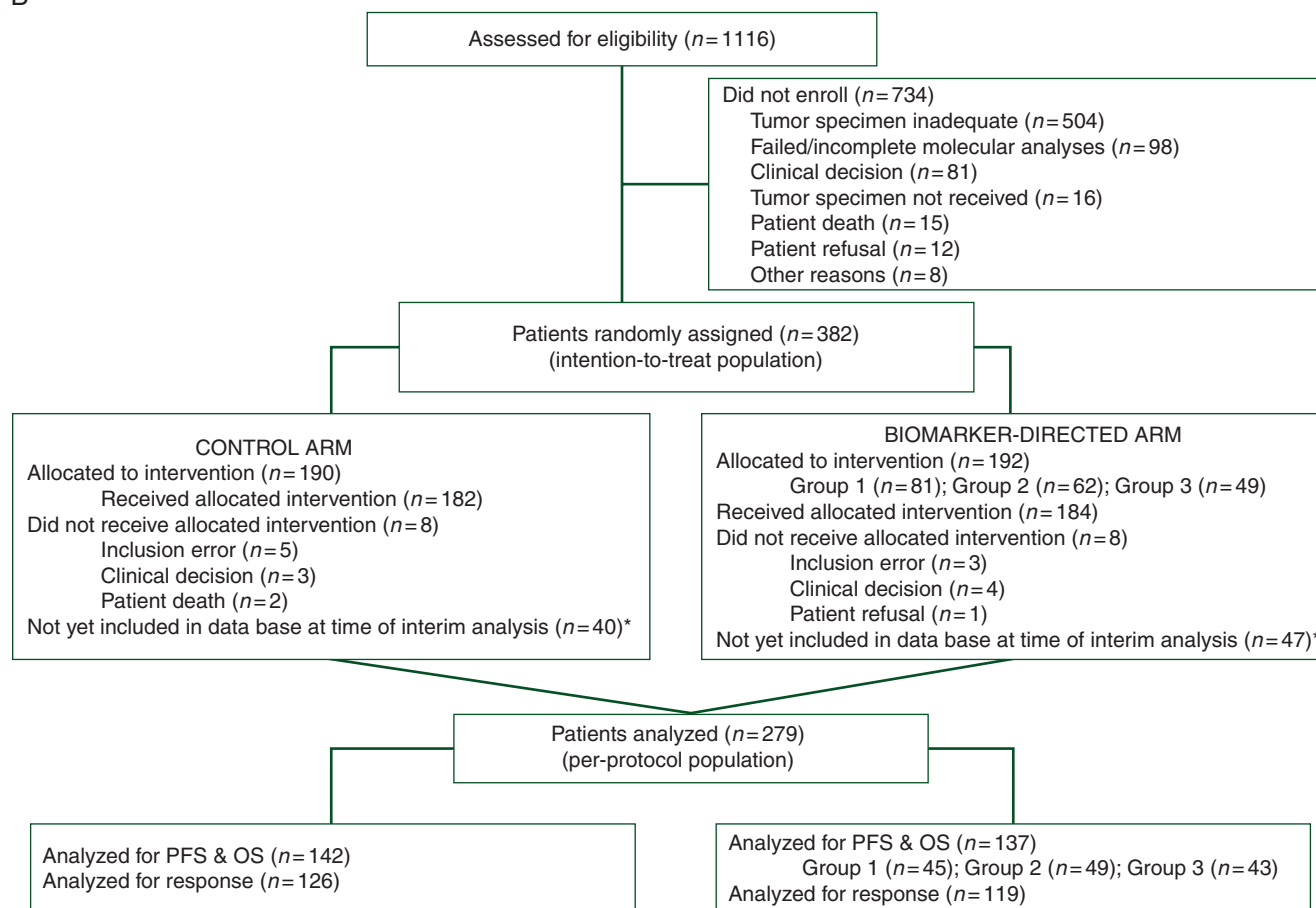
intermediate or high RAP80 expression and high BRCA1 expression (group 3) received docetaxel (75 mg/m^2) on day 1. All cycles were 21 days.

Patients in the Chinese trial were randomized in a 1 : 3 ratio to either the control or the experimental arm. Treatment regimens were the same as in the SLCG study, but dosages were adjusted to standard doses for the Chinese population (docetaxel 60–75 mg/m^2; gemcitabine 1000 mg/m^2).

molecular analysis

BRCA1 and RAP80 gene expression were analyzed in RNA isolated from paraffin-embedded tumor tissues. A hematoxylin/eosin-stained slice was examined by the pathologist to select the tumor area. In Spain, two 4-μm slices were mounted on special slides (Pem-Membrane slides, Palm, Oberlensheim, Germany) for laser capture microdissection (CAPmover Microdissector, Carl Zeiss Microimaging, Barcelona, Spain) to ensure a minimum of 90% of tumor cells. In China, two 5-μm-thick slices per block with at least 10 mm^2 of tumor area were macrodissected to ensure a minimum of 80% of tumor cells.

B

Assessed for eligibility (*n*=1116)

Did not enroll (*n*=734)
    Tumor specimen inadequate (*n*=504)
    Failed/incomplete molecular analyses (*n*=98)
    Clinical decision (*n*=81)
    Tumor specimen not received (*n*=16)
    Patient death (*n*=15)
    Patient refusal (*n*=12)
    Other reasons (*n*=8)

Patients randomly assigned (*n*=382)
(intention-to-treat population)

CONTROL ARM
Allocated to intervention (*n*=190)
        Received allocated intervention (*n*=182)
Did not receive allocated intervention (*n*=8)
        Inclusion error (*n*=5)
        Clinical decision (*n*=3)
        Patient death (*n*=2)
Not yet included in data base at time of interim analysis (*n*=40)*

BIOMARKER-DIRECTED ARM
Allocated to intervention (*n*=192)
        Group 1 (*n*=81); Group 2 (*n*=62); Group 3 (*n*=49)
Received allocated intervention (*n*=184)
Did not receive allocated intervention (*n*=8)
        Inclusion error (*n*=3)
        Clinical decision (*n*=4)
        Patient refusal (*n*=1)
Not yet included in data base at time of interim analysis (*n*=47)*

Patients analyzed (*n*=279)
(per-protocol population)

Analyzed for PFS & OS (*n*=142)
Analyzed for response (*n*=126)

Analyzed for PFS & OS (*n*=137)
        Group 1 (*n*=45); Group 2 (*n*=49); Group 3 (*n*=43)
Analyzed for response (*n*=119)

*These patients were randomized shortly before the interim analysis was performed and had not yet been included in the data base.

Fig. 1 *Continued*

RNA extraction, retro transcription and real-time PCR analysis were carried out as described in the Appendix. Specific primers and probe for each gene expression were designed according to the Ref Seq (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene) using Primer Express 2.0 Software (Applied Biosystems, Foster City, CA) (supplementary Table S1, available at *Annals of Oncology* online). Quantification of gene expression was carried out using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems). (Further details are provided in supplementary Material, available at *Annals of Oncology* online.)

statistical analysis

In the phase III SLCG study, we estimated that 372 events would be needed for the study to have a power of 90% to detect a hazard ratio (HR) of 1.50 (median PFS of 7.8 months in the experimental versus 5.2 months in the control arm), using a two-sided log-rank test with significance set at 0.01. With a constant enrollment over 15 months and assuming an overall loss of 10% after randomization, a total of 480 patients would need to be randomized. An interim analysis was scheduled when 50% of progression events (186) had occurred.

In the phase II Chinese study, assuming an exponential model for PFS, a total of 72 events would be required for the study to have a power of 80% to detect a HR of 2.80. With a constant enrollment over 14 months and

assuming a 5% loss after randomization, a total of 124 subjects would need to be randomized in the Chinese trial.

PFS and OS were estimated with the Kaplan–Meier method and compared with a two-sided log-rank test. A Cox regression analysis was used to calculate HRs with their 95% confidence intervals (CIs), both raw and adjusted for random stratification factors. Response rates were calculated using the Pearson-Clopper method. Differences between baseline characteristics and treatment arms were compared with either the two-sided Fisher's exact test or the χ^2 test for categorical variables and with the student's *t*-test for age.
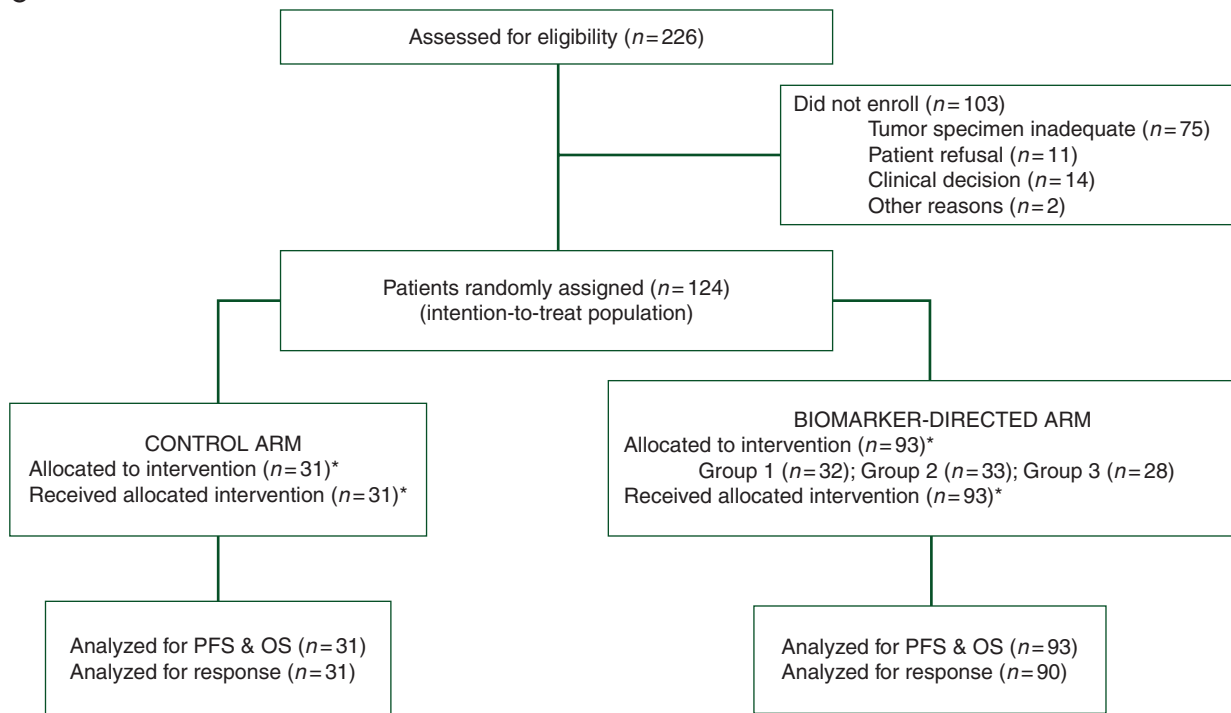
All analyses were two-sided with a 5% significance level and were carried out with SPSS version 19 and SAS version 9.2. (Complete details are shown in supplementary Material, available at *Annals of Oncology* online.)

results

patients and treatment

In the SLCG randomized phase III trial, 1116 patients were assessed for eligibility. At the time of data cutoff (15 October 2012), 382 had been randomized, 279 of whom were included in the per-protocol population: 142 in the control arm and 137 in the experimental arm (Figure 1B). Baseline characteristics were well balanced between the two arms with the exception of sex

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│   Assessed for eligibility (n = 226) │
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Did not enroll (n = 103)
    Tumor specimen inadequate (n = 75)
    Patient refusal (n = 11)
    Clinical decision (n = 14)
    Other reasons (n = 2)

Patients randomly assigned (n = 124)
(intention-to-treat population)

CONTROL ARM
Allocated to intervention (n = 31)*
Received allocated intervention (n = 31)*

BIOMARKER-DIRECTED ARM
Allocated to intervention (n = 93)*
    Group 1 (n = 32); Group 2 (n = 33); Group 3 (n = 28)
Received allocated intervention (n = 93)*

Analyzed for PFS & OS (n = 31)
Analyzed for response (n = 31)

Analyzed for PFS & OS (n = 93)
Analyzed for response (n = 90)

*All patients received the allocated intervention and were eligible for analysis; hence 124 were included in the per-protocol population.

Fig. 1 *Continued*

(P = 0.05) (Table 1, supplementary Table S2, available at *Annals of Oncology* online).

In the Chinese randomized phase II trial, 226 patients were assessed for eligibility. At the time of data cutoff (10 July 2013), 124 had been randomized—31 to the control arm and 93 to the experimental arm (Figure 1C). Baseline characteristics were well balanced between the two arms (Table 1, supplementary Table S2, available at *Annals of Oncology* online).

Information on EGFR mutations is provided in supplementary Material, available at *Annals of Oncology* online.

progression-free survival

In the SLCG trial, of the 279 patients included in the per-protocol population, 215 (58% of planned events) had progressed or died at the time of the interim analysis. PFS was 5.3 months (95% CI 4.71–5.88) for all patients, 5.49 months (95% CI 5.08–5.91) for the control arm and 4.38 months (95% CI 3.27–5.48) for the experimental arm (log rank P = 0.07; raw HR for progression in the experimental arm 1.28; 95% CI 0.98–1.67; P = 0.07; adjusted HR 1.35; 95% CI 1.02–1.78; P = 0.03) (Figure 2A). PFS was 5.43 months in group 1, 5.49 months in group 2, and 2.50 months in group 3 (log rank P for comparison of control arm and three experimental groups = 0.003). A harmful effect was observed for group 3 (patients with high/intermediate RAP80 and high BRCA1 levels receiving single-agent docetaxel) compared with the control arm (adjusted HR, 2.65; 95% CI 1.66–4.24; P < 0.001) (Figure 2B, supplementary Table S3, available at *Annals of Oncology* online).

Of the 124 patients in the per-protocol population of the Chinese trial, 112 had progressed or died at the time of this analysis. PFS was 3.91 months (95% CI 2.8–5.03) for the entire cohort, 4.74 months (95% CI 1.97–7.5) for the control arm and 3.78 months (95% CI 2.52–5.04) for the experimental arm (log rank P = 0.82; raw and adjusted HRs, 0.95; P = 0.82) (Figure 2C). PFS was 5.59 months for group 1, 3.78 months for group 2, and 12.73 months for group 3 (log rank P for comparison of control arm and three experimental groups = 0.55) (Figure 2D, supplementary Table S3, available at *Annals of Oncology* online).

overall survival

In the SLCG trial, OS was 10.16 months (95% CI 8.32–12.01) for all patients, 12.66 months (95% CI 10.07–15.26) in the control arm and 8.52 months (95% CI 6.41–10.63) in the experimental arm (log rank P = 0.006; raw HR, 1.55; 95% CI 1.13–2.12; P = 0.006; adjusted HR, 1.85; 95% CI 1.33–2.57; P < 0.001). A harmful effect was again observed for group 3 (adjusted HR, 2.54; 95% CI 1.49–4.34; P = 0.001) (supplementary Table S3 and Figure S1a and b, available at *Annals of Oncology* online).

In the Chinese trial, OS was 11.74 months (95% CI 7.94–15.55) for all 124 patients, 10.82 months (95% CI 2.32–19.33) in the control arm and 11.74 months (95% CI 8.06–15.43) in the experimental arm (log rank P = 0.94; raw HR 0.98; 95% CI 0.57–1.69; P = 0.94; adjusted HR 0.99; 95% CI 0.57–1.76; P = 0.99) (supplementary Table S3 and Figure S1c and d, available at *Annals of Oncology* online).

Table 1. Baseline characteristics of patients included in the per-protocol populations in the SLCG and Chinese studies

SLCG study (N = 279)

	All patients (N = 279), n (%)	Control arm (N = 142), n (%)	Experimental arm (N = 137), n (%)	P
Age (years)				
Median (range)	62 (37–83)	62 (42–82)	63 (37–83)	0.37
Sex				
Female	52 (18.6)	33 (23.2)	19 (13.9)	0.05
Male	227 (81.4)	109 (76.8)	118 (86.1)	
ECOG PS				
0	93 (33.3)	44 (31)	49 (35.8)	0.23
1	186 (66.7)	98 (69)	88 (64.2)	
Histology				
Adenocarcinoma	142 (50.9)	74 (52.1)	68 (49.6)	0.89
Large cell carcinoma	23 (8.2)	10 (7)	13 (9.5)	
Squamous cell carcinoma	99 (35.5)	50 (35.2)	49 (35.8)	
Other	15 (5.4)	8 (5.6)	7 (5.1)	
Smoking status				
Current smoker	74 (27.8)	38 (26.8)	36 (26.3)	0.71
Former smoker	179 (67.3)	89 (62.7)	90 (65.7)	
Never smoked	13 (4.9)	8 (5.6)	5 (3.7)	
Data unavailable	13 (4.9)	7 (4.9)	6 (4.4)	
Number of cycles				
Median (range)	4 (1–8)	5 (1–8)	4 (1–7)	0.30
Second-line treatment				
Yes	133 (47.7)	73 (51.4)	60 (43.8)	0.23
No	146 (52.3)	69 (48.6)	77 (56.2)	

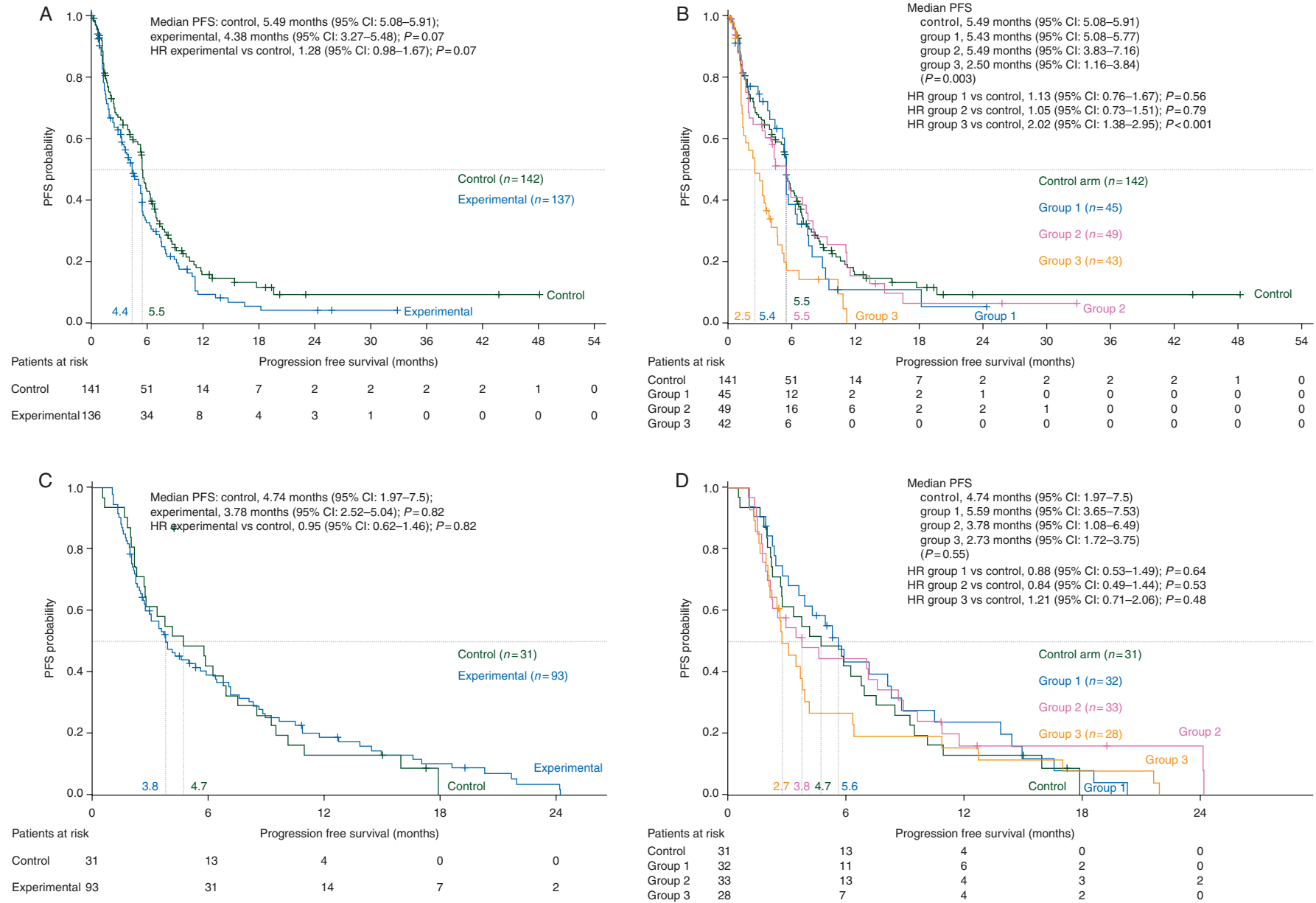|	Chinese study (N = 124)			
	All patients (N = 124), n (%)	Control arm (N = 31), n (%)	Experimental arm (N = 93), n (%)	P
Age (years)				
Median (range)	59 (28–78)	59 (31–74)	59 (28–78)	0.51
Sex				
Female	35 (28.2)	8 (25.8)	27 (29)	0.82
Male	89 (71.8)	23 (74.2)	66 (71)	
ECOG PS				
0	30 (24.2)	5 (16.1)	25 (26.9)	0.33
1–2	94 (75.8)	26 (83.9)	68 (73.1)	
Histology				
Squamous cell carcinoma	42 (33.9)	11 (35.5)	31 (33.3)	0.83
Nonsquamous cell carcinoma	82 (66.1)	20 (64.5)	62 (66.7)	
Smoking status				
Current/former smoker	77 (62.1)	21 (67.7)	56 (60.2)	0.53
Never smoked	47 (37.9)	10 (32.3)	37 (39.8)	
Number of cycles				
Median (range)	4 (1–6)	4 (1–6)	4 (1–6)	0.54
Second-line treatment				
Yes	68 (54.8)	17 (54.8)	51 (54.8)	0.99
No	56 (45.2)	14 (45.2)	42 (45.2)	

other secondary end points

Information on response and toxicities is provided in supplementary Material and Table S4, available at *Annals of Oncology* online.

subgroup analyses

Additional exploratory subgroup analyses of PFS were carried out in the SLCG trial using stratification and prognostic variables to investigate the potential differential effect of clinical factors on outcome according to treatment arm. Complete details are shown in supplementary Material and Table S5 and Figure S2, available at *Annals of Oncology* online. In addition, given the markedly poor outcome observed in group 3 of the biomarker-directed arm (single-agent docetaxel), we compared this group of 43 patients with the 34 patients in the control arm having the same gene expression profile (high/intermediate RAP80 and

Figure 2. Progression-free survival. (A) In the SLCG study by treatment arm; (B) in the SLCG study comparing the control arm and each of the three biomarker-directed groups; (C) in the Chinese study by treatment arm; and (D) in the Chinese study comparing the control arm and each of the three biomarker-directed groups. Raw HRs are shown. HRs adjusted for stratification factors [histology (squamous versus non-squamous); ECOG PS (0 versus 1); RAP80 expression (low versus intermediate versus high); BRCA1 expression (low versus intermediate versus high)] can be found in Results section and in supplementary Table S3, available at *Annals of Oncology* online.

high BRCA1 expression). A significant benefit was observed for patients in the control arm compared with those in the experimental arm (PFS = 6.38 versus 2.5 months; log rank $P < 0.0001$; HR 2.62; $P = 0.001$). PFS for patients with the other two gene expression profiles did not differ significantly between the two arms (supplementary Table S6, available at *Annals of Oncology* online).

discussion

The SLCG conducted a randomized, international, phase III trial in patients with advanced NSCLC with the objective of examining whether biomarker-directed chemotherapy based on BRCA1 and RAP80 expression levels would confer improved PFS compared with nonselected cisplatin-based chemotherapy. At the same time, a randomized phase II study was carried out in China under the auspices of the SLCG to ascertain whether the predictive role of BRCA1 and RAP80 expression observed in European patients would also hold true among Chinese patients. Our expectation was that our findings would validate previous data indicating a combinatory effect of BRCA1 and RAP80 expression on PFS and OS [10, 12]. However, the prespecified interim analysis of the SLCG study showed a detrimental effect in the biomarker-directed arm (HR for progression 1.35; $P = 0.03$) and the SLCG phase III randomized trial was prematurely closed.

The detrimental effect observed in the experimental arm was especially marked in group 3, where patients received single-agent docetaxel (HR 2.65; $P < 0.001$). One limitation of this study is that it is not possible to analyze the predictive role of the biomarkers since the two arms did not receive the same treatment, nor can we compare the effect of the different treatment regimens since they were administered to different patient populations. At the time of designing the studies, it was expected that patients with intermediate/high RAP80 and high BRCA1 expression would benefit from single-agent docetaxel, based on previous findings that BRCA1 expression induces sensitivity to antimicrotubule agents [6, 7] and that RAP80 regulates BRCA1 function [4], as well as on the results of the SLCG phase II trial [10]. However, since an exploratory analysis found a benefit for patients with this gene expression profile in the control arm, who received docetaxel/cisplatin, compared with the same group of patients in the experimental arm, who received docetaxel ($P < 0.001$), we can speculate that the detrimental effect in the experimental arm may well have been partly due to the use of single-agent docetaxel. This potential harmful effect of monotherapy should be kept in mind when designing future clinical trials. One of the few previous studies comparing docetaxel with docetaxel/cisplatin as first-line treatment was carried out in unselected patients and reported a higher response rate for the combination regimen, but this did not translate into significantly longer PFS or OS [16].

Among the other two gene expression groups, there was no difference in PFS between the control and experimental arms, which may indicate that the predictive capacity previously reported for RAP80 expression [4, 10] seems to be only part of the complex molecular network influencing the BRCA1 model. BRCA1 plays a central but still enigmatic role in homologous recombination, and as part of the genetic analyses specified as a secondary end point in the BREC protocol, we are now examining alternative biomarkers that could elucidate DNA repair mechanisms, including p53-binding protein 1 (53BP1) [17, 18] and other DNA damage response factors, such as RING finger protein 8 (RNF8) and RNF168 E3 ubiquitin ligases [19] (supplementary Figure S3, available at *Annals of Oncology* online).

The BREC studies were sponsored by the SLCG in collaboration with the FLCG and received no support from the pharmaceutical industry. Although 504 patients were not eligible for inclusion due to inadequate tumor specimens, this had been foreseen in the protocol, and the BREC studies provide proof of concept that an international, nonindustry, biomarker-directed trial is feasible. Moreover, the centralization of the gene expression analyses eliminated any potential interinstitutional variations in specimen processing and analysis. In addition, our unexpected negative findings highlight the importance of close clinical validation of preclinical findings before undertaking a major randomized clinical trial. We expect that thanks to the groundwork laid by the BREC studies, further research can help to define predictive models for chemotherapy outcome and contribute to therapeutic approaches of synthetic lethality [20, 21].

disclosure

The authors have declared no conflicts of interest.

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appendix