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ORIGINAL ARTICLE

Carboplatin plus pemetrexed for platinum-pretreated, advanced non-small cell lung cancer: a retrospective study with pharmacogenetic evaluation

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Abstract
Purpose In the present study, the combination of carboplatin (CBDCA) plus pemetrexed (PMX) for the treatment of patients with platinum-pretreated, pemetrexed-naïve, advanced non-small cell lung cancer (NSCLC) was investigated. Also, single nucleotide polymorphisms (SNPs) at the *XRCC3*, *XPD*, *ERCC1*, *GARFT*, *DHFR*, and *TS* genes were investigated.
Methods Eighty patients treated with CBDCA/PMX at two Italian institutions were evaluable. Of these, 73 patients had blood samples collected for pharmacogenetic evaluation.
Results Overall, the median age was 59 years (26–78), 59 patients (73.7%) had a performance status of 0, and 34 patients (42.5%) had stage IIIB disease. Thirty-eight patients (47.5%) had responded to prior first-line platinum-based therapy. Study treatment resulted into one complete and 33 partial responses for an overall response rate of 42.5%. The disease control rate was 77.5%. The median progression-free survival (PFS) and overall survival (OS) were 5.8 and 17.4 months, respectively. Responders achieved a significant longer PFS and OS versus non-responders ($P = 0.007$ and $P = 0.003$, respectively). The only grade 3–4 adverse event occurring in more than 5.0% of patients was neutropenia (6 patients, 7.5%). No statistically significant association was noted between polymorphisms of the genes analyzed and clinical outcome.
Conclusions In patients with platinum-pretreated, advanced NSCLC and favorable clinical prognostic factors, treatment with CBDCA/PMX is associated with a good clinical outcome and toxicity profile. None of the SNPs analyzed was found to be a useful predictor of treatment efficacy.

Keywords Carboplatin · Non-small cell lung cancer · Pemetrexed · Single nucleotide polymorphisms · Disease relapse · Salvage chemotherapy

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Introduction

Lung cancer is among the most commonly diagnosed cancers worldwide, representing the first cause of cancer-related deaths in both the USA and Europe [1, 2]. Non-small cell lung cancer (NSCLC) accounts for approximately 80% of all lung cancers being often diagnosed at an advanced stage when treatment options are limited. First-line treatment of patients with advanced disease is generally platinum-based, yielding a median overall survival of 8–11 months [3]. This poor survival outcome has been only marginally extended

when a targeted agent was added to a platinum-based chemotherapy backbone [4, 5].

Second-line treatment is indicated for patients who are still in good performance status and willing to be treated further despite disease progression to front-line therapy. The aims of second-line treatment consist mainly in symptoms palliation and, possibly, survival improvement [6–9]. At the present time docetaxel, the multitarget antifolate pemetrexed and the epidermal growth factor receptor tyrosine kinase inhibitor erlotinib are the only drugs registered for use as single agents in this setting [6–9]. However, in spite of the superiority of single agent over best supportive care in second-line NSCLC, the prognosis of these patients remains poor with a median survival of 6–8 months. For this reason, patients candidate to second-line treatments may potentially benefit from a combination therapy that includes re-challenging with a platinum agent [10]. Particularly, because of their additive antitumor activity, the cytotoxic combination of carboplatin plus pemetrexed (CBDCA/PMX) represents an appealing salvage strategy for platinum-pretreated advanced NSCLC patients [11]. Moreover, two phase II studies investigating CBDCA/PMX as first-line treatment in advanced NSCLC patients showed response rates in the range of 24 and 31.6% and 1-year survival of 56 and 43.9%, respectively, with a very good toxicity profile [12, 13].

Polymorphic variants in DNA repair genes can explain inter-individuals differences in sensitivity to anticancer-agents. The importance of identifying single nucleotide polymorphisms (SNPs) that influence chemotherapy outcome would allow physicians to deliver effective treatments to sensitive patients, while preventing others from suffering the side effects of inactive drugs. On the one hand, SNPs of genes involved in DNA double-strand break/recombination repair might account for sensitivity to platinum compounds [14]. On the other, SNPs of genes intercalated in the metabolism of pemetrexed could account for increased sensitivity to this agent [15].

On this basis, we undertook an observational analysis of platinum-pretreated, pemetrexed-naïve, advanced NSCLC patients who were administered CBDCA/PMX as salvage chemotherapy at two Italian institutions looking also at SNPs of *XRCC3*, *XPD*, *ERCC1*, *GARFT*, *DHFR*, and *TS* genes.

Patients and methods

Using a prospectively maintained database including patients treated for advanced NSCLC (stage IIIB or IV) at two Italian institutions (Azienda Ospedaliera S. Maria della Misericordia in Perugia; Istituto Oncologico del Mediterraneo in Catania), we identified those individuals who had received salvage chemotherapy with CBDCA/PMX. In order to render the study results updated with the most recent TNM staging of NSCLC, the seventh edition of tumor staging was used to class patients stages [16]. Eighty out of 489 patients were identified and selected for the present analysis based on the following criteria: pretreatment with first-line platinum-based chemotherapy, no more than 3 previous lines of therapy received, no prior pemetrexed, disease progression following the most recent treatment prior to CBDCA/PMX, presence of measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST) criteria [17], treatment with at least two cycles of CBDCA/PMX, Eastern Cooperative Oncology Group performance status (ECOG PS) $\leq$2, and adequate renal, hepatic and hematologic function prior to initiation of CBDCA/PMX. Patients with asymptomatic and/or controlled brain metastases were considered eligible for the analysis regardless of whether they had received appropriate radiation therapy to the brain.

All patients had signed a written informed consent prior to CBDCA/PMX, and the present retrospective analysis was approved by the Institutional Review Boards of both institutions in which it was conducted. At the time of treatment initiation, patients were offered to participate to a pharmacogenetic study by signing a separate informed consent for the collection of baseline blood samples in order to evaluate SNPs of a few pre-specified genes.

Treatment schedule

All patients received pemetrexed 500 mg/m^2 administered intravenously as a bolus infusion of 10 min followed by carboplatin area under the curve (AUC) 5 according to the Calvert formula given as an intravenous infusion of 30 min, both drugs administered on day 1 of a 21-day cycle. Starting approximately 1 week before day 1 of cycle 1, all patients received supplementation with folic acid (400 μg daily orally until 3 weeks after discontinuation of study treatment) and vitamin B12 (1,000 μg intramuscularly every 9 weeks throughout the duration of treatment) in order to reduce the pemetrexed-related hematologic toxicity. Treatment was usually given until disease progression, unacceptable toxicity, or withdrawal of the patient. However, the treating physician could choose anytime either to stop treatment in the absence of disease progression or continue treatment with pemetrexed monotherapy.

Response and toxicity evaluation

Pretreatment evaluation included medical history and physical examination, complete hematology, blood chemistry, electrocardiography, chest X-ray, and tumor measurement based on standard radiologic methods. Brain

imaging was performed if brain metastases were suspected clinically. During the treatment period, hematology and blood chemistry were performed every cycle. Tumor assessments were performed every three cycles, and disease response was categorized as complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) according to the RECIST criteria [17]. Toxicity was scored every 3 weeks according to the Common Toxicity Criteria of the National Cancer Institute (NCI-CTC, version 3.0) [18]. For the toxicity analysis, the worst data for each patient from all the chemotherapy cycles were used.

DNA extraction and SNP genotyping

Genomic DNA was extracted from 200 µl of whole blood samples using the QIAamp DNA extraction kit on Biorobot EZ1 instrument (Qiagen, Milan, Italy) according to the manufacturer's instructions. The nine SNPs located in 7 genes *XRCC3* Thr241Met (C/T); *XPD* Lys751Gln (A/C); *ERCC1* Asn/118Asn (C/T); *GARFT* C/G; *DHFR* C/G, *DHFR* A/G were detected with TaqMan-probe-based assays and *TYMS* 5′-UTR and *TYMS* 3′-UTR with PCRs followed by RFLP as previously described by other authors [14, 19, 20]. The PCR reactions were done using 20 ng of genomic DNA diluted in 11.875 µl Dnase-Rnase free water, 12.5 µl of TaqMan Universal PCR Master Mix with AmpliTaq Gold, and 0.625 µl of the assay mix (forward- and reverse-specific primers and the specific probes), in a total volume of 25 µl. Amplification was done under the following conditions: 50°C for 2 min., 95°C for 10 min. followed by 40 cycles of 92°C for 15 s and 60°C for 1 min. Fluorescence in each sample well was measured before and after PCR using ABI Prism 7300 Sequence Detection System. Data were analyzed using the Allelic Discrimination Program (Applied Biosystems, Foster City, CA). For each polymorphism, a minimum of 20 randomly selected DNA samples were genotyped at least twice to confirm the results.

Statistical analysis

Demographic and clinical information were compared across genotype using Pearson's chi-square or Fisher exact test as appropriate. Progression-free survival (PFS) was the time elapsed from the date of initiation of CBDCA/PMX to the date of first evidence of progression or death of the patient in the absence of documented disease progression. The overall survival (OS) time was measured from the start of CBDCA/PMX to the date of death for any cause. In the absence of death, OS was censored at the time of the last visit. Patients without an event were censored at the date of last follow-up (August 2010). Time to event (PFS and OS) was analyzed according to Kaplan–Meier method, and survival curves were compared using the log-rank test. Cox model was used to estimate hazard ratio and related 95% confidence interval. Given the retrospective nature of the study, statistical significance should be used in an exploratory view and median time estimation with their 95% confidence interval reported to better interpret the data. SPSS software version 17.0 was used for statistical analyses (SPSS Inc., Chicago, IL).

Results

Patient characteristics

From December 2006 to December 2009, a total of 80 patients were treated with CBDCA/PMX as second, third, or fourth line of therapy. The baseline characteristics are listed in Table 1. The median age was 59 years (range 26–78), and 49 patients (61.3%) were male. Nearly three-quarters of patients ($N = 59$, 73.7%) had an ECOG PS of 0, and 20 patients (25.0%) were never smokers. Adenocarcinoma histology was present in 58 patients (72.5%), and 34 patients (42.5%) had stage IIIB disease, of which 5/34 (14.7%) were candidate to receive chemotherapy concomitant with radiotherapy. Forty-six patients (57.5%) received CBDCA/PMX as second-line whereas 34 patients (42.5%) as third or fourth line.

Response and survival

Overall, twenty-two patients (27.5%) received less than 6 cycles of CBDCA/PMX. Of the remainder 58 patients (72.5%) who were not progressing at the sixth cycle of therapy, 26 (44.8%) were delivered ≥1 additional cycle of chemotherapy (either CBDCA/PMX, $N = 17$ or PMX monotherapy $N = 11$). The median number of cycles of CBDCA/PMX was 6 (range 2–12). Table 2 shows response to treatment. One patient obtained a complete response, and 33 patients achieved a partial response for an overall response rate of 42.5%. The overall disease control rate was 77.5%. At the time of the analysis (August 2010), 76 patients (95.0% of the total) have progressed, of which 54 patients (71.0%) died. After a median follow-up of 15 months (range 3–44), the median PFS was 5.8 months (95% CI: 4.8–6.7), and the median OS was 17.4 months (95% CI: 14.1–20.6; Fig. 1a, b). No statistically significant difference in terms of response, PFS, and OS was noted according to histology (adenocarcinoma vs. squamous), line of therapy (second vs. third or fourth line), and time elapsed since termination of first-line platinum-based chemotherapy (≥6 months vs. <6 months). By contrast, a

Table 1 Patients and tumor characteristics

	N = 80
Median age, years (range)	59 (26–78)
Gender	
Male	49 (61.3%)
Female	31 (38.7%)
ECOG PS	
0	59 (73.7%)
1	19 (23.8%)
2	2 (2.5%)
Smoking history	
Never smoker[a]	20 (25.0%)
Former smoker[b]	30 (37.5%)
Current smoker	30 (37.5%)
Stage	
IIIB	34 (42.5%)
IV	46 (57.5%)
Histology	
Adenocarcinoma	58 (72.5%)
Squamous cell carcinoma	13 (16.3%)
Unclassified	9 (11.2%)
Number of prior lines of therapy	
1	46 (57.5%)
2	26 (32.5%)
3	8 (10.0%)
Prior first-line chemotherapy	
Cisplatin-gemcitabine	55 (68.8%)
Carboplatin-gemcitabine	10 (12.5%)
Carboplatin-paclitaxel	8 (10.0%)
Cisplatin-etoposide	2 (2.5%)
CCRT with cisplatin-gemcitabine	5 (6.2%)
Prior biological therapy	
EGFR-TKI[c]	35 (43.7%)
None	45 (56.3%)
Best response to first-line platinum-based therapy	
Complete response	3 (3.8%)
Partial response	35 (43.7%)
Stable disease	22 (27.5%)
Progressive disease	15 (18.8%)
Not evaluable	5 (6.2%)
Time elapsed since termination of first-line platinum-based therapy	
≥6 months	63 (78.8%)
<6 months	17 (21.2%)
Median progression-free survival of first-line platinum-based therapy, months (95% CI)	6.5 (5.4–7.7)

CCRT chemotherapy concomitant with radiotherapy, *ECOG PS* Eastern Cooperative Oncology Group performance status, *EGFR-TKI* epidermal growth factor receptor-tyrosine kinase inhibitor

[a] <100 cigarettes in a lifetime

[b] ≥100 cigarettes in a lifetime; stopped smoking within the last year of diagnosis

[c] Either erlotinib or gefitinib as monotherapy

Table 2 Response and survival

	N = 80
Response	
Complete response	1 (1.2%)
Partial response	33 (41. 3%)
Stable disease	28 (35.0%)
Progressive disease	18 (22.5%)
Overall response	34 (42.5%)
(95% CI)	(31.7–53.3)
Disease control[a]	62 (77.5%)
Median duration of response, months (range)	6.9 (4–30+)
1-year survival rate	64.5%
2-year survival rate	32.0%
Median follow-up, months (range)	15 (3–44)

[a] Overall response + stable disease

statistically significant difference in terms of median PFS was noted between responders and non-responders (Fig. 1c). Similarly, the median OS was significantly longer for responding patients compared with non-responders (Fig. 1d).

Toxicity

All patients were evaluable for toxicity. Treatment-related adverse events are listed in Table 3 (all grades, maximum toxicity per patient reported). With the exception of hematologic toxicity, the frequency of treatment-related toxicity exceeding CTCAE grade 2 was less than 5.0% for all categories. The most common grade 3–4 hematological adverse event was neutropenia occurring in 7.5% of patients. However, only half of these cases (3.7%) were complicated with hospitalization for febrile neutropenia.

Post-progression treatment

Forty-seven patients (58.7%) received at least one line of post-progression therapy consisting of docetaxel and a tyrosine kinase inhibitor (either erlotinib or gefitinib) in the majority of cases (36.1 and 31.9%, respectively). The median number of post-progression lines of treatment was 2 (range 1–5).

Gene polymorphisms

Table 4 reports the frequency of the polymorphisms of the genes analyzed. All polymorphisms followed Hardy–Weinberg's equilibrium. However, no statistically significant association was noted between certain genotypes and clinical or pathologic characteristics. Similarly, no significant association was found with response to treatment, PFS, or

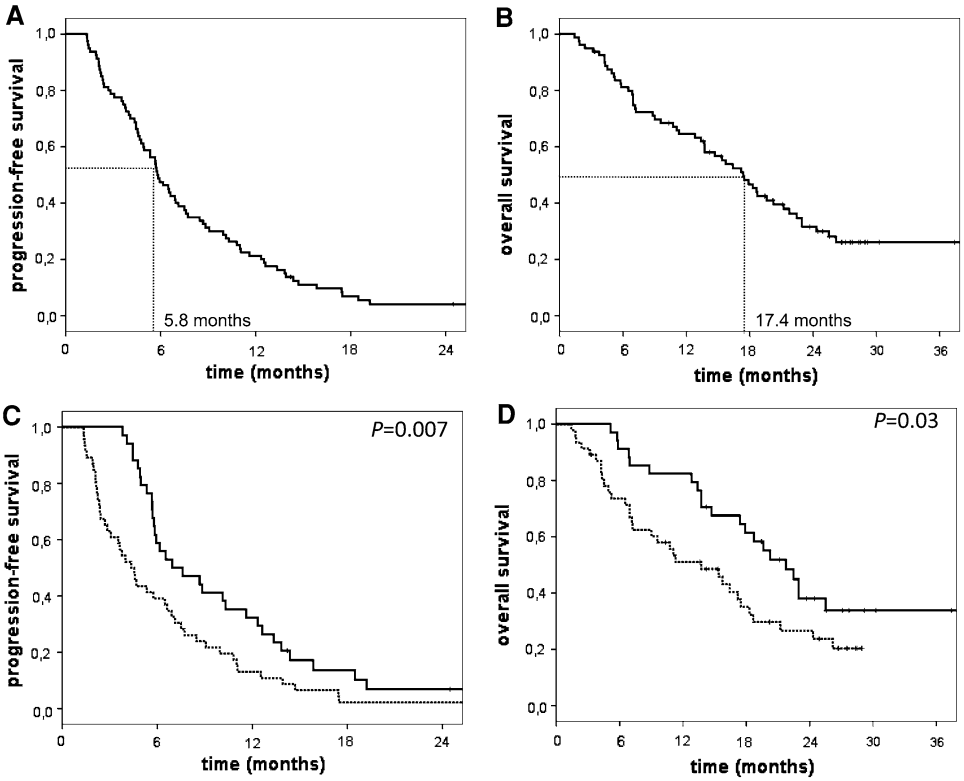

Fig. 1 Kaplan–Meier estimates of median progression-free survival (*PFS*) (**a**) and median overall survival (*OS*) (**b**) for all patients. Kaplan–Meier estimates of median PFS (**c**) and OS (**d**) according to response to treatment. Patients experiencing a complete or partial response (N = 34) had a significantly longer PFS and OS compared with non-responders ($N = 46$) (6.9 months vs. 4.3 months for PFS, respectively, $P = 0.007$; 21.7 months vs. 13.7 months for OS, respectively, $P = 0.03$)

Table 3 Summary of treatment-related adverse events

	$N = 80$			
	Grade 1	Grade 2	Grade 3	Grade 4
Hematological/laboratory adverse events				
Leucopenia	10 (12.5%)	4 (5.0%)	3 (3.7%)	1 (1.2%)
Neutropenia	6 (7.5%)	3 (3.7%)	4 (5.0%)	2 (2.5%)
Thrombocytopenia	3 (3.7%)	2 (2.5%)	3 (3.7%)	1 (1.2%)
Anemia	6 (7.5%)	1 (1.2%)	1 (1.2%)	1 (1.2%)
Hypercreatininemia	–	–	1 (1.2%)	–
Non-hematological adverse events				
Nausea/vomiting	10 (12.5%)	6 (7.5%)	1 (1.2%)	–
Dyspepsia	5 (6.2%)	2 (2.5%)	–	–
Mucositis	5 (6.2%)	1 (1.2%)	1 (1.2%)	–
Rash	5 (6.2%)	1 (1.2%)	1 (1.2%)	–
Fatigue	6 (7.5%)	2 (2.5%)	–	–

OS. Also, when focusing only on the non-squamous subgroup of patients, no significant association was noted between certain genotypes with patients characteristics or clinical outcome.

As the incidence of grade 3 and 4 toxicities was low, genotypes were tested for their association with any chemotherapy-related toxicity. No association between genotypes and chemotherapy-related toxicity was found.

Discussion

The current study was carried out in order to evaluate the combination of CBDCA/PMX in patients with platinum-pretreated advanced NSCLC. Intriguingly, although comparison across studies is likely to be biased by the existence trial-specific differences in terms of inclusion criteria/patients characteristics, the clinical outcome reported with CBDCA/PMX in the present study appears favorable when compared with other studies available in the literature (Table 5) [21–23]. Notably, the present results appear even more intriguing if we consider that nearly half of patients had been pretreated with ≥2 lines of therapy.

Recently, two phase II randomized trials have compared CBDCA/PMX with PMX monotherapy in NSCLC patients failing a first-line platinum-based chemotherapy [21, 22]. Of these, only the trial by Smit et al. showed a greater percentage of responses for CBDCA/PMX over PMX monotherapy (17.0% vs. 6.0%, respectively) with a statistically significant prolongation of PFS favoring the combination regimen (4.2 months vs. 2.8 months, respectively, $P = 0.005$) [21]. Notably, in our study, the response rate to CBDCA/PMX was found to be more than doubled compared with that observed by Smit et al. (Table 5), which translated into median PFS and OS values of 5.8 and 17.4 months, respectively. Nevertheless, a few considerations need to be carried

Table 4 XRCC3, XPD, ERCC1, GARFT, DHFR C/G, DHFR A/G, TYMS 5′-UTR, TYMS 3′-UTR polymorphisms

Genotype	No. pts = 70
XRCC3	
TT	18 (24.7%)
TC	38 (52%)
CC	17 (23.3%)
XPD	
AA	31 (42.5%)
AC	26 (35.6%)
CC	16 (21.9%)
ERCC1	
CC	25 (34.2%)
CT	35 (48%)
TT	13 (17.8%)
GARFT	
CC	40 (54.8%)
CG	28 (38.4%)
GG	5 (6.8%)
DHFR C/G	
CC	29 (39.8%)
CG	33 (45.2%)
GG	11 (15%)
DHFR A/G	
GG	50 (68.5%)
AG	22 (30.1%)
AA	1 (1.4%)
TYMS 5′-UTR	
2R/2R	14 (19.2%)
2R/3R	34 (46.6%)
3R/3R	25 (34.2%)
TYMS 3′-UTR	
6+/6+	23 (31.5%)
6+/6−	36 (49.3%)
6−/6−	14 (19.2%)

out in order to put our favorable results into context. First, our population was undoubtedly selected for positive clinical characteristics since it included a great percentage of patients with PS 0, stage IIIB disease and responders to prior first-line platinum-based chemotherapy, all features that have been clearly proven to be independent prognostic factors for longer survival in advanced NSCLC patients receiving second-line chemotherapy [24]. Moreover, the fact that nearly half of the patients of our analysis had responded to prior first-line platinum-based chemotherapy might have helped select a population particularly sensitive to a chemotherapeutic regimen that includes re-challenging with a platinum agent. To this regard, a recent retrospective study of 28 advanced NSCLC patients responding to first-line chemotherapy showed that subsequent re-challenge with the same chemotherapeutic regimen used in the first-line can lead to a median survival of 17 months and a 1-year survival of 60% [25]. Interestingly, such survival values are very close to those observed with CBDCA/PMX in our platinum-pretreated population (17.4 months for median OS and 64.5% for 1-year survival). Finally, subsequent post-progression treatments might have positively influenced our favorable survival results. In fact, more than half of patients received at least one further line of therapy for whom a median number of 2 subsequent lines of treatment was reported.

When it comes to salvage therapy, safety always represents a relevant issue. In the present study, CBDCA/PMX confirmed to be a very well-tolerated regimen when given as salvage chemotherapy to platinum-pretreated patients. Particularly, the only grade 3–4 adverse event observed in more than 5% of patients was neutropenia (6 patients, 7.5%; Table 3). This excellent tolerability might justify the use of CBDCA/PMX as salvage chemotherapy in fit patients, especially considering that the improved outcome reported for responding patients did not come at the expense of an excess in toxicity (Fig. 1c, d).

With regard to pharmacogenetic investigations, unlike others [21], we were not able to show any significant correlation between the polymorphisms of the genes analyzed and clinical outcome to CBDCA/PMX. Nevertheless, a number of NSCLC studies have been conducted with the aim of correlating SNPs of certain genes, particularly ERCC1 and XPD, with the activity of a given therapy, often reporting conflicting results in this end-point [20, 26–30]. For instance, the ERCC1 C/C phenotype has been correlated with longer survival in platinum-treated advanced NSCLC patients in two studies [26, 27], whereas no association was found in others [20, 28]. Similarly, in our study, we did not report any significant difference in survival according to the ERCC1 polymorphism ($P = 0.87$, by log-rank test), with median survival times of 17.9 (C/C), 17.5 (C/T), and 13.3 (T/T) months, respectively (data not shown). The contradictory reports on the results of ERCC1 and other gene polymorphisms may be likely explained by the great heterogeneity of patients and type of tumors analyzed among different studies. Also, another potential explanation that can be put forward for the negative findings observed in NSCLC pharmacogenetic studies including ours might consist in the fact that the measurement of genotype in blood samples may not truly reflect the genotype of tumor tissue. In fact, although literature studies suggest a high concordance rate for certain gene polymorphisms between paired tumor and non-diseased tissue or blood samples [31–33], it could be faulty to assume that such concordance will be present for all genes of interest in pharmacogenetic or molecular epidemiologic studies as

Table 5 Studies evaluating the combination of carboplatin plus pemetrexed as salvage chemotherapy for platinum-pretreated NSCLC

Author	Type of study	Line of therapy	No. of pts	RR (%)	DCR (%)	PFS (mos.)	OS (mos.)
Smit et al. [21]	Randomized phase II	2nd	119	17.0	72.0	4.2	8
Tiseo et al. [22]	Randomized phase II	2nd	119	12.6	60.5	3.5	9
Kim et al. [23]	Uncontrolled phase II	2nd and 3rd	32	18.7	46.8	2.3	9.4
Current study	Retrospective	2nd to 4th	80	42.5	77.5	5.8	17.4

DCR disease control rate, *mos.* months, *OS* overall survival, *PFS* progression-free survival, *RR* response rate

well as in the specific tumor types of interest. For these reasons, future studies evaluating specific SNPs for advanced NSCLC should be carried out in a prospective manner and whenever possible, it may be preferable to use diseased samples to assess the utility of the SNPs that are relevant to a particular research question.

In conclusion, salvage chemotherapy with CBDCA/PMX was associated with an excellent clinical outcome and a very good toxicity profile in platinum-pretreated, pemetrexed-naïve, advanced NSCLC patients harboring favorable clinical prognostic factors. Prospective studies of chemotherapy in advanced NSCLC should keep exploring pharmacogenetics in order to identify group of patients carrying biological characteristics associated with clinically meaningful variations in drug responsiveness.

Conflict of interest None declared.

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