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SHORT REPORT

Reduced folate carrier (RFC) as a predictive marker for response to pemetrexed in advanced non-small cell lung cancer (NSCLC)

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Summary *Introduction* RFC is the major transport system in mammalian cells for folate cofactors and antifolate therapeutics. The aim of this study was to assess the predictive value of RFC expression in patients receiving pemetrexed for advanced NSCLC. *Methods* The study was carried out in a population of 48 patients with advanced NSCLC which have received pemetrexed monotherapy in second and third line. RFC expression was assessed using a two-step model of immunohistochemical staining in paraffin-embedded tissue samples. *Results* RFC expression was detected in 16 (33 %) patients. In the global population, the median progression free survival (PFS) and the median overall survival (OS) were 3.3 and 6.5 months respectively. The subgroup of patients with expression of RFC had a tendency to better median PFS (4.5 vs 2.8 months; $p=0.926$) and median OS (11.7 vs 4.8; $p=0.150$). In patients with adenocarcinoma histology and RFC expression median OS after treatment with pemetrexed was 14.4 months versus 5.0 in those with adenocarcinoma but without RFC expression ($p=0.039$). *Conclusions* These results suggest the possible relation between RFC expression and response to treatment with antifolates (pemetrexed) independently of the tumor histology. Further studies are required to confirm these results.

Keywords Reduced folate carrier · Pemetrexed · Advanced non-small-cell lung cancer · Predictive marker

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Introduction

Lung cancer has the highest incidence of all cancers worldwide, with 1.35 million cases diagnosed each year (12.4 % of all new cancers); it also has the highest number of deaths, at some 1.18 million deaths (17.6 % of all cancer deaths worldwide) [1]. Non-small-cell lung cancer (NSCLC) accounts for some 85 % of all lung cancers diagnoses; most people diagnosed with this histology are unsuitable for surgery, as they present with advanced disease at the time of diagnosis. Guideline recommendations include platinum-based combinations as first-line treatment in suitable patients [2, 3], which results in response rates of 20–40 % and median overall survival of 7–12 months. Efforts to improve treatment outcomes have identified differences in survival that depend on tumor histology; specifically, treatment with pemetrexed in patients with non-squamous NSCLC exhibited greater efficacy than gemcitabine (both in combination with cisplatin) [4], docetaxel (both as single-drug and second-line therapies) [5], or placebo (as maintenance therapy) [6] or as early second-line treatment [7].

Pemetrexed is a multi-target antifolate that inhibits five different enzymes in the folate pathway: thymidylate synthase (TS), dihydrofolate reductase (DHFR), glycinamide ribonucleotide transformylase (GARTF), and, to a lesser extent, aminoimidazole carboxamide ribonucleotide transformylase (AICARTF), and CI-tetrahydrofolate synthase. This mechanism depletes fully reduced folate, causing disruption of nucleotide synthesis from both pyrimidines and purines [8, 9]. Of these enzymes TS is the most widely studied as a predictor of response to pemetrexed. High TS expression appears to confer reduced sensitivity to pemetrexed [10, 11].

Pemetrexed and other synthetic antifolates are designed to be taken up efficiently by the reduced folate carrier (RFC),

the primary transport system of both tetrahydrofolate (THF) cofactors and antifolates. RFC is a ubiquitously expressed ~85 kDa membrane glycoprotein with 12 transmembrane segments, and cytoplasmically located N- and C-termini. RFC is a classic member of the solute carrier (SLC) family of facilitative carriers. RFC lacks a nucleotide-binding domain (NBD) and is therefore considered a low-capacity transporter. Defective antifolate influx is widely associated with decreased human RFC gene expression and down-regulation of the transporter protein in various preclinical studies with different antifolate-resistant cell lines [12].

With all that in mind, we hypothesized that response to antifolate treatment with pemetrexed in advanced NSCLC might be related to RFC expression in tumor cells.

Material and methods

The predictive value of RFC expression was retrospectively analyzed in a cohort of patients with advanced-stage NSCLC treated in our department. The inclusion criteria for the study were as follows: patients treated with pemetrexed in second- or third-line (previous treatment with tyrosine kinase inhibitors was allowed), availability of sufficient tumor tissue for immunohistochemical analysis, and accessible clinical data. RFC expression was measured at the Pathology Department of the Hospital Universitario Central de Asturias. Clinical data were collected from patient files.

RFC expression was assessed using a two-step model of immunohistochemical staining. Formalin-fixed, paraffin-embedded (FFPE) tissues were obtained from all examined patients. Laboratory measurement of RFC was performed using RFC-1 (C-17: sc-47357; Santa Cruz Biotechnology, Inc.), an affinity-purified goat polyclonal antibody against the RFC C-terminus. Immunohistochemical staining was carried out at room temperature using a humidity chamber, followed by incubation with a high-sensitivity detection kit, following the manufacturer's instructions. The sections were dewaxed with xylene, rinsed in graded ethanol, and rehydrated in water before blocking endogenous peroxidase activity with 3 % H2O2 for 10 min. Antigen retrieval was achieved by heating the slices in 0.01 M citrate buffer (pH 6.0), at room temperature. An FFPE section of human choriocarcinoma with antibody dilution served as a positive control for RFC staining.

Pemetrexed was administered at 500 mg/m2 of body surface area (BSA) as an intravenous infusion over 10 min on the first day of each 21-day cycle in association with folinic acid and vitamin B12 supplements. Response or non-response to treatment was evaluated every two cycles or whenever disease progression was suspected, applying RECIST v1.0 criteria (chest X-ray and thoracoabdominopelvic computed tomography).

Progression free survival (PFS) and overall survival (OS) were evaluated using the Kaplan–Meier methodology and Log-rank test. Multivariate analyses assessed the effects of performance status (PS), sex, smoking, histology, and RFC expression on PFS and OS.

Results

From July 2005 through July 2010, 48 patients with advanced NSCLC were treated with pemetrexed. The main characteristics of these patients are shown in Table 1. Of these 48 patients, 29 received pemetrexed as second line (54.2 %) and 19 as third line (39.6 %). At the time of analysis, 45 patients (93.8 %) had died and only three patients were still alive (6.2 %, data censored).

Median PFS and OS of the population following treatment with pemetrexed were 3.3 months (95 % CI: 0.1–6.6) and 6.5 months (95 % CI: 2.1–10.2), respectively, without any difference between patients treated as second- and third-line. Because of the similar outcomes, the data were pooled for analysis to achieve greater statistical power.

RFC expression was detected in 16 patients (33.3 %; 14 adenocarcinomas, 1 squamous cell carcinoma, and 1 patient with mixed histology -predominantly squamous cell carcinoma). Lack of RFC expression was observed in 32 patients (66.7 %; 25 adenocarcinoma and 7 squamous cell carcinoma). Clinical characteristics of the population by RFC expression are shown in Table 2.

Table 1 Baseline characteristics of the patients

		N	%
Age (*median*, *in years*)	56 (42–75)		
Sex			
	Male	33	68.75
	Female	15	31.25
Smoking			
	Yes	23	47.92
	No	10	20.83
	Former smoker	15	31.25
Performance status (ECOG)			
	0–1	37	77.08
	≥2	11	22.92
Histology			
	Adenocarcinoma	39	81.25
	Squamous cell carcinoma	8	16.67
	Mixed	1	2.08
RFC expression			
	Yes	16	33.33
	No	32	66.67

Table 2 Clinical characteristics of the study population according to RFC expression

		Expression of RFC	Lack of RFC expression
Age (*median, in years*)		55 (42–72)	59 (42–75)
Sex			
	Male	10 (30.30 %)	23 (69.70 %)
	Female	6 (40 %)	9 (60 %)
Smoking			
	Yes	4 (17.39 %)	19 (82.61 %)
	No	3 (30 %)	7 (70 %)
	Former smoker	9 (60 %)	6 (40 %)
Performance status (ECOG)			
	0–1	14 (37.84 %)	23 (62.16 %)
	≥2	2 (18.18 %)	9 (81.82 %)
Histology			
	Adenocarcinoma	14 (35.90 %)	25 (64.10 %)
	Squamous cell carcinoma	1 (12.50 %)	7 (87.50 %)
	Mixed	1	0

PFS and OS showed no statistically significant differences with respect to gender, histology, or RFC expression. Among patients with better PS (PS 0–1), PFS (4.1 vs 1.4 months, $P<0.001$) and OS (8.7 vs 1.4 months, $P<0.001$) were significantly longer than for those with PS 2. Non-smoking patients at the time of diagnosis had significantly better OS (11.7 vs 3.9-months, $P=0.001$), but showed no difference in PFS (Table 3).

Table 3 Median progression free survival (PFS) and median overall survival (OS) in months

	Median PFS (*months*)	P-value	Median OS (*months*)	P-value
Sex		0.055		0.273
Male	3.1		6.5	
Female	4.2		5.5	
Smoking		0.105		0.001
Yes	2.9		3.9	
No	4.2		11.7	
Performance status (ECOG)		<0.001		<0.001
0–1	4.1		8.7	
≥2	1.4		1.4	
Histology		0.620		0.762
Adenocarcinoma	3.6		7.4	
Squamous cell carcinoma	2.4		2.9	
RFC expression		0.926		0.150
Yes	4.5		11.7	
No	2.8		4.9	

Table 4 Median overall survival (OS) depending on histology and RFC expression

Histology and expression of RFC	Median OS (*months*)	P-value
		0.004
Adenocarcinoma positive for RFC expression	14.4	
Adenocarcinoma negative for RFC expression	5.0	
Squamous cell carcinoma negative for RFC expression	4.9	

For patients with adenocarcinoma histology and RFC expression in tumor samples, median OS after treatment with pemetrexed was 14.4 months versus 5.0 in those with adenocarcinoma but no RFC expression ($P=0.039$). Median OS was similar in patients without RFC expression, irrespective of histology: five months for adenocarcinomas and 4.9 months for squamous cell carcinomas ($P=0.004$; Table 4 and Fig. 1).

Discussion

Pemetrexed has been approved in the EU and US (and elsewhere) as initial therapy in combination with cisplatin [4], and as monotherapy following chemotherapy [13] and maintenance treatment [7] in patients with predominantly non-squamous NSCLC, which is considered to be a predictive factor of efficacy by this drug. Other independent factors that affect survival prognosis, apart from histology (adenocarcinoma vs squamous cell carcinoma), include disease stage, performance status, race, and sex [4].

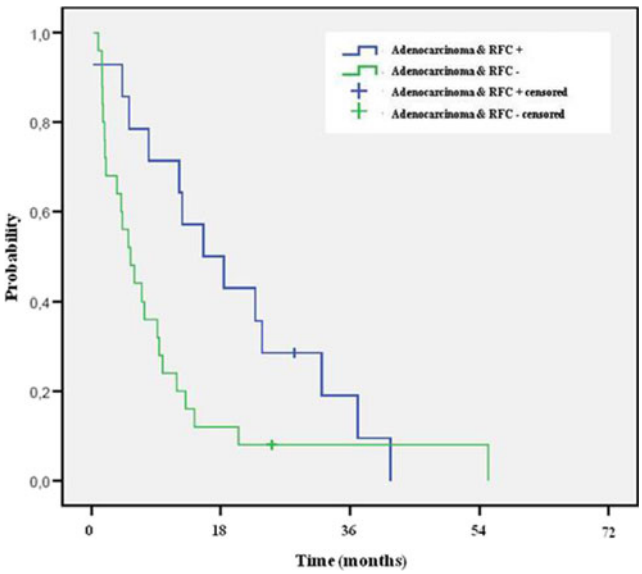

Fig. 1 Overall survival of adenocarcinomas treated with pemetrexed depending on RFC expression. ($P=0.039$)

Pemetrexed is a potent TS inhibitor, and is a weaker inhibitor of other enzyme targets (DHFR, GARTF, and AICARTF). The differences in survival that favor pemetrexed in patients with non-squamous NSCLC histology may be explained by the observation that higher TS mRNA and protein levels have been found in squamous cell and high-grade carcinomas resected from chemotherapy-naive patients with NSCLC [14].

The ubiquitously expressed RFC is the major transport system for classical antifolate therapeutics. Loss of RFC expression or function can have profound pathophysiological consequences. For chemotherapeutic antifolates used for cancer, such as methotrexate or pemetrexed, loss of RFC proteins results in antifolate resistance due to incomplete inhibition of cellular enzyme targets [15]. Decreased RFC expression has been detected in acute lymphoblastic leukemia (ALL), osteosarcoma, and colorectal cancer, as well as primary central nervous system (CNS) lymphoma at the transcript and/or protein levels. Impaired transport of antifolates into tumor cells in preclinical and clinical studies has been associated with quantitative and/or qualitative alterations in RFC expression and/or transport [16, 17].

The relationship between RFC expression in NSCLC and treatment outcomes with pemetrexed has not been widely studied. Recently, however, polymorphisms in the RFC gene have appeared to predict survival differences in pemetrexed-treated NSCLC [18].

In our study, patients with a favorable PS at diagnosis, female sex, non-smoker status, and adenocarcinoma subtype exhibited better PFS and OS outcomes, which are consistent with previously reported data [6, 13]. Moreover, RFC expression was detected in 33.3 % of the population studied. Reduced expression was applicable in smokers and squamous cell carcinoma. In contrast, RFC expression was detected more frequently in adenocarcinomas. No significant differences were observed regarding sex or PS in RFC expression.

Overall, RFC expression is associated with longer median PFS and OS, particularly in patients with adenocarcinoma histology, where the median OS was 14.4 months when RFC was expressed versus 5 months for those patients with no expression of RFC (P=0.039). When tumor samples show absence of RFC, PFS and OS are similar regardless of histology.

Taking into account the limitations of this retrospective study and sample size, these data suggest that the clinical benefit attributable to pemetrexed is related to the expression of the carrier that introduces the drug inside the tumor cell, rather than tumor histology. Adenocarcinomas with high RFC expression may have better outcomes than those with no RFC expression. Although seeing what benefit could be achieved by using pemetrexed in squamous cell carcinoma with high RFC expression would be intriguing, finding out would be difficult, as pemetrexed has no indication in squamous cell carcinomas.

These results emphasized the need for better patient selection, which should be based on both histological characteristics and on molecular differences.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P (2005) Global cancer statistics, 2002. CA Cancer J Clin 55(2):74–108
2. D'Addario G, Fruh M, Reck M, Baumann P, Klepetko W, Felip E (2010) Metastatic non-small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 21(Suppl 5):v116–v119
3. Azzoli CG, Temin S, Aliff T, Baker S Jr, Brahmer J, Johnson DH, Laskin JL, Masters G, Milton D, Nordquist L, Pao W, Pfister DG, Piantadosi S, Schiller JH, Smith R, Smith TJ, Strawn JR, Trent D, Giaccone G (2011) 2011 focused update of 2009 American Society of Clinical Oncology Clinical Practice Guideline Update on chemotherapy for stage IV non-small-cell lung cancer. J Clin Oncol 29(28):3825–3831
4. Scagliotti GV, Parikh P, von Pawel J, Biesma B, Vansteenkiste J, Manegold C, Serwatowski P, Gatzemeier U, Digumarti R, Zukin M, Lee JS, Mellemgaard A, Park K, Patil S, Rolski J, Goksel T, de Marinis F, Simms L, Sugarman KP, Gandara D (2008) Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. J Clin Oncol 26(21):3543–3551
5. Scagliotti G, Hanna N, Fossella F, Sugarman K, Blatter J, Peterson P, Simms L, Shepherd FA (2009) The differential efficacy of pemetrexed according to NSCLC histology: a review of two Phase III studies. Oncologist 14(3):253–263
6. Ciuleanu T, Brodowicz T, Zielinski C, Kim JH, Krzakowski M, Laack E, Wu YL, Bover I, Begbie S, Tzekova V, Cucevic B, Pereira JR, Yang SH, Madhavan J, Sugarman KP, Peterson P, John WJ, Krejcy K, Belani CP (2009) Maintenance pemetrexed plus best supportive care versus placebo plus best supportive care for non-small-cell lung cancer: a randomised, double-blind, phase 3 study. Lancet 374(9699):1432–1440
7. Paz-Ares L, de Marinis F, Dediu M, Thomas M, Pujol JL, Bidoli P, Molinier O, Sahoo TP, Laack E, Reck M, Corral J, Melemed S, John W, Chouaki N, Zimmermann AH, Visseren-Grul C, Gridelli C (2012) Maintenance therapy with pemetrexed plus best supportive care versus placebo plus best supportive care after induction therapy with pemetrexed plus cisplatin for advanced non-squamous non-small-cell lung cancer (PARAMOUNT): a double-blind, phase 3, randomised controlled trial. Lancet Oncol 13(3):247–255
8. Gridelli C, Maione P, Rossi A, Bareschino MA, Schettino C, Sacco PC, Zeppa R (2011) Pemetrexed in advanced non-small cell lung cancer. Expert Opin Drug Saf 10(2):311–317
9. Esteban E, Casillas M, Cassinello A (2009) Pemetrexed in first-line treatment of non-small cell lung cancer. Cancer Treat Rev 35(4):364–373
10. Sun JM, Han J, Ahn JS, Park K, Ahn MJ (2011) Significance of thymidylate synthase and thyroid transcription factor 1 expression in patients with nonsquamous non-small cell lung cancer treated with pemetrexed-based chemotherapy. J Thorac Oncol 6(8):1392–1399
11. Takezawa K, Okamoto I, Okamoto W, Takeda M, Sakai K, Tsukioka S, Kuwata K, Yamaguchi H, Nishio K, Nakagawa K (2011)

Thymidylate synthase as a determinant of pemetrexed sensitivity in non-small cell lung cancer. Br J Cancer 104(10):1594–1601
12. Assaraf YG (2007) Molecular basis of antifolate resistance. Cancer Metastasis Rev 26(1):153–181
13. Hanna N, Shepherd FA, Fossella FV, Pereira JR, De Marinis F, von Pawel J, Gatzemeier U, Tsao TC, Pless M, Muller T, Lim HL, Desch C, Szondy K, Gervais R, Shaharyar, Manegold C, Paul S, Paoletti P, Einhorn L, Bunn PA Jr (2004) Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. J Clin Oncol 22(9):1589–1597
14. Ceppi P, Volante M, Saviozzi S, Rapa I, Novello S, Cambieri A, Lo Iacono M, Cappia S, Papotti M, Scagliotti GV (2006) Squamous cell carcinoma of the lung compared with other histotypes shows higher messenger RNA and protein levels for thymidylate synthase. Cancer 107(7):1589–1596
15. Matherly LH, Hou Z, Deng Y (2007) Human reduced folate carrier: translation of basic biology to cancer etiology and therapy. Cancer Metastasis Rev 26(1):111–128
16. Wettergren Y, Odin E, Nilsson S, Willen R, Carlsson G, Gustavsson B (2005) Low expression of reduced folate carrier-1 and folylpolyglutamate synthase correlates with lack of a deleted in colorectal carcinoma mRNA splice variant in normal-appearing mucosa of colorectal carcinoma patients. Cancer Detect Prev 29(4):348–355
17. Ferreri AJ, Dell'Oro S, Capello D, Ponzoni M, Iuzzolino P, Rossi D, Pasini F, Ambrosetti A, Orvieto E, Ferrarese F, Arrigoni G, Foppoli M, Reni M, Gaidano G (2004) Aberrant methylation in the promoter region of the reduced folate carrier gene is a potential mechanism of resistance to methotrexate in primary central nervous system lymphomas. Br J Haematol 126(5):657–664
18. Adjei AA, Salavaggione OE, Mandrekar SJ, Dy GK, Ziegler KL, Endo C, Molina JR, Schild SE (2010) Correlation between polymorphisms of the reduced folate carrier gene (SLC19A1) and survival after pemetrexed-based therapy in non-small cell lung cancer: a North Central Cancer Treatment Group-based exploratory study. J Thorac Oncol 5(9):1346–1353