Radiotherapy and Oncology 119 (2016) 229–235

Contents lists available at ScienceDirect

Radiotherapy and Oncology

journal homepage: www.thegreenjournal.com

Phase II trial

Plasma ceramide, a real-time predictive marker of pulmonary and hepatic metastases response to stereotactic body radiation therapy combined with irinotecan

Nolwenn Dubois [a,b,c,d], Emmanuel Rio [a], Natacha Ripoche [b,c,d], Véronique Ferchaud-Roucher [c,e], Marie-Hélène Gaugler [b,c,d], Loic Campion [a], Michel Krempf [c,e], Christian Carrie [f], Marc Mahé [a], Xavier Mirabel [g], François Paris [a,b,c,d,*]

[a] Institut de Cancérologie de l'Ouest, Saint-Herblain; [b] Inserm, UMR892, Nantes; [c] Université de Nantes; [d] CNRS, UMR 6299; [e] INRA, UMR 1280, Nantes; [f] Centre Léon Bérard, Lyon; and [g] Centre Oscar Lambret, Lille, France

ARTICLE INFO

Article history:
Received 19 August 2015
Received in revised form 15 March 2016
Accepted 18 March 2016
Available online 21 April 2016

Keywords:
Biomarker
Ceramide
Stereotactic radiotherapy
Irinotecan
Metastases

ABSTRACT

Background and purposes: Early biomarkers of tumour response are needed to discriminate between responders and non-responders to radiotherapy. We evaluated the ability of ceramide, a bioactive sphingolipid, to predict tumour sensitivity in patients treated by hypofractionated stereotactic body radiation therapy (SBRT) combined with irinotecan chemotherapy.

Materials and methods: Plasma levels of total ceramide and of its subspecies were measured before and during treatment in 35 patients with liver and lung oligometastases of colorectal cancer included in a phase II trial. Cer levels were quantified by LC–ESI-MS/MS and compared to tumour volume response evaluated one year later by CT-scan.

Results: Pretreatment plasma ceramide levels were not indicative of tumour response. Nevertheless, the levels of total ceramide and of its 4 main subspecies were significantly higher at days 3 and 10 of treatment in objective responders than in non-responders. According to Kaplan–Meier curves, almost complete tumour control was achieved at 1 year in patients with increased total ceramide levels whereas 50% of patients with decreased levels experienced an increase in tumour volume.

Conclusions: Total plasma ceramide is a promising biomarker of tumour response to SBRT combined with irinotecan that should enable to segregate patients with high risk of tumour escape.

Radiation therapy is a common palliative and curative anticancer treatment but its efficacy is limited by intrinsic tumour resistance to radiation [1]. The development of stereotactic body radiation therapy (SBRT) has led to better tumour targeting and to the delivery of higher radiation doses in a limited number of fractions [2]. However, the efficacy of SBRT in reducing oligometastases and small solid tumours needs to be assessed for all tumours depending upon its resistance, size and location, usually by measuring tumour volume by non-invasive imaging techniques, such as CT-scan, MRI, or PET-scan. Since the response of the tumour is generally detected long after the end of radiotherapy, with late radiological modifications after SBRT being particularly difficult to assess [3], there is a real risk of prolonged unnecessary and ineffective exposure to radiation and of delays in initiating alternative treatments.

The availability of biomarkers that distinguish between responding and refractory patients early during the course of radiotherapy would represent a major clinical advance to define patients with high risk of tumour escape to the treatment. A potentially interesting biomarker is ceramide (Cer), a pro-apoptotic sphingolipid generated rapidly after irradiation. Cer is produced in the outermost layer of the cell membrane on hydrolysis of sphingomyelin by acidic sphingomyelinase (ASM) or neutral sphingomyelinase (NSM) and is synthetized *de novo* in endoplasmic reticulum by Cer synthase [4]. Adding exogenous Cer to androgen-sensitive human prostate adenocarcinoma cells (LNCAP cells) enhanced cell radiosensitivity and tumour regression [5]. Increasing endogenous Cer in human T lymphocyte cells (Jurkat cells) by the action of inhibitors of glucosyl-Cer synthase and ceramidase inhibitors (DL-PDMP and D-MAPP, respectively)

* Corresponding author at: Centre de Recherche en Cancérologie Nantes-Angers UMR Inserm 892 CNRS 6299, 8 quai Moncousu, 44007 Nantes, France.
E-mail address: francois.paris@inserm.fr (F. Paris).

http://dx.doi.org/10.1016/j.radonc.2016.03.014
0167-8140/

also enhanced cell radiosensitivity [6]. Cer induced by high dose irradiation provoked massive endothelial cell apoptosis via ASM activation, and regression of fibrosarcoma or melanoma transplanted in wild-type mice [7].

Elevated Cer levels have been observed in plasma and serum from patients with lung emphysema [8], Wilson disease [9] and multiple organ failure [10]. Plasma Cer levels increased during lipid infusion in humans and rats, and were correlated with insulin sensitivity, inflammation and atherosclerotic risk [11]. Increased Cer serum levels were recorded after 3 days of treatment in 5 of 7 patients who responded to spatial fractionated grid radiotherapy (SFGRT) including a first irradiation at 15 Gy followed by 30×2 Gy [12]. However, no robust correlation was established between Cer and radiotherapy efficacy because of the small number of patients (11 in total) and the diversity of tumours.

During a phase II trial of hypofractionated SBRT combined with irinotecan chemotherapy, we measured plasma Cer levels in patients with liver and lung oligometastases originating from primary colon tumours. The objective was to validate plasma Cer as an early biomarker of treatment efficacy by correlating variations in Cer levels during treatment with long-term tumour response.

Materials and methods

Patient eligibility

Biological samples were collected from an ancillary study of a phase II clinical trial (EudraCT 2006-005440-87; clinical trial NCT01220063) performed from 2008 to 2013 by 3 French oncology centres (Nantes, Lyon and Lille). The main objective of the phase II clinical study (Table 1) was to test the feasibility of SBRT in combination with irinotecan chemotherapy for colorectal adenocarcinoma lung and liver metastases. The clinical follow-up is still ongoing and will be published separately.

Treatment planning and delivery

A phase II clinical trial (EudraCT 2006-005440-87; clinical trial NCT01220063) of SBRT and concomitant irinotecan chemotherapy for colorectal adenocarcinoma lung and liver metastases was performed from 2008 to 2013 in 3 oncology centres in France (Nantes, Lyon and Lille). Patient inclusion criteria were inoperable or recurrent hepatic and/or lung metastases after surgery, relapse after fluorouracil treatment with or without eloxatin or irinotecan, life expectancy >6 months, measurable metastases (largest diameter $\leqslant$6 cm, sum of the maximum diameter of multiple metastases $\leqslant$6 cm), a clinical target volume (CTV) located more than 12 mm laterally or 15 mm in the cranio-caudal direction of the stomach, small intestine, oesophagus, trachea, and pulmonary arteries, and an adequate haematologic cell pool and adequate hepatic and renal functions to receive irinotecan. Exclusion criteria were a performance index >2 (World Health Organization scale), prior thoraco-abdominal irradiation, a contraindication to irinotecan, prior (within 5 years) or concomitant treatment of an invasive cancer, diffuse metastatic disease, or more than 3 metastases. The ethics committees of all 3 institutions approved the protocol, and signed informed consent was obtained from all patients.

Table 1
Patient characteristics and demography.

		Location	
		Lung	Liver
Patient (number)	Male	8	21
	Female	1	5
Age (year)	Median	65	66.5
	Youngest	32	33
	Oldest	77	84
Tumour diameter (mm)	Median	13	36
	Smallest	4	11
	Largest	26	100

Treatment protocol is described in Fig. S1. Radiotherapy consisted in 4 fractions of 10 Gy delivered on days (D) 1, 3, 8 and 10 using linear accelerator-based devices (Novalis, Brain Lab, Feldkirchen, Germany, or Cyberknife, Accuray, Sunnyvaley, CA). In each case, 99% of the CTV was encompassed by the 75–95% isodose lines corresponding to a 42–53 Gy dose at the target centre. Irinotecan (40 mg/m^2) (Pfizer, New York, NY) was intravenously injected 30–90 min before delivery of the first and third radiotherapy fractions.

Response criteria

As decided in 2008, tumour response was assessed, using RECIST 1.1 (Response Evaluation Criteria In Solid Tumors) on chest or liver CT-scans, 3, 6, and 12 months post-treatment [13]. A complete response (CR) was defined by the disappearance of all target lesions, a partial response (PR) as a >30% decrease in the sum of the largest diameter (LD) of target lesions, progressive disease (PD) as a >20% increase of the LD of each lesion, and stable disease (SD) as neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD.

Plasma biocollection

Blood samples (20 ml) were collected in sodium citrate tubes before the first (D0) and after 30 min the second (D3) and fourth (D10) fractions (Fig. S1), stored at 4 °C for 30 min, then centrifuged at 1000 g for 5 min at 4 °C. The plasma aliquots were stored at −80 °C until analysis.

Cer extraction, purification and analysis

Ultrapure standards of 12 Cer subspecies (C14:0, C16:1, C16:0, C18:1, DHC18:0, C18:0, C20:1, C20:0, C22:1, C22:0, C24:1 and C24:0) and non-natural C17:0 Cer used as an internal standard were purchased from Avanti Polar Lipids (Alabaster, AL). UPLC grade methanol and analytical grade organic solvents were purchased from Fisher Scientific (Pittsburgh, PA). Forty microlitres of 1 μM C17:0 Cer were added to each plasma sample. Lipids were extracted from 100 μl of plasma samples in two steps using a previously described procedure with minor modifications [14]: (1) addition of 1.5 ml of a acidified hexane/propan-2-ol mixture (60:40, v/v), vortexing, centrifugation at 3000*g* for 5 min at 4 °C and collection of the upper phase; (2) addition of 1.5 ml of acidified methanol, homogenization, centrifugation at 8000*g* for 5 min at 4 °C, and collection of the upper phase. The organic phases from steps 1 and 2 were combined, dried under nitrogen at room temperature and resuspended in 150 μl of hexane/propan-2-ol (60:40 v/v).

The lipid extract was purified using an optimized published method [15]. Briefly, samples were loaded on 100-mg LC-NH_2 cartridges (Interchim, Montluçon, France) preconditioned with 2 ml of hexane. The cartridges were washed first with 1.4 ml of ethyl acetate–hexane 15:85 (v/v) to elute neutral lipids in a single fraction, then with 1.6 ml of chloroform/methanol 23:1 (v/v) to elute free Cer. The Cer fraction was dried under nitrogen and dissolved in 300 μl of MeOH containing 10 mM highest grade ammonium acetate (Fluka, Buchs, Switzerland) and 0.2% formic acid. Samples were stored at −20 °C until analysis.

Purified Cer fractions were analysed by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC–ESI-MS/MS) on an Acquity H-Class UPLC system combined with a Xevo

TQD triple quadrupole mass spectrometer (Waters Corporation, Milford, CT). Gradient chromatographic separation was performed on a Waters C18 BEH column (2.1 mm × 50 mm, 1.8 μM particle size, 0.5 μM prefilter, column heater at 43 °C). The mobile phases consisted of MiliQ water containing 0.2% formic acid and 10 mM ammonium acetate (Eluent A) and methanol containing 0.2% formic acid and 10 mM ammonium acetate (Eluent B). The injection volume was 5 μl. Purified Cer were eluted in 4 min with a linear gradient of 95–98% of eluent B (flow rate: 0.6 ml/min). Before the next elution, the column was re-equilibrated from 4.00 to 4.10 min and stabilized from 4.10 to 6 min with 95% of eluent B. All analyses were performed using electrospray ionization in the positive ion mode with multiple reactions monitoring. Data were acquired and analysed by Mass-Lynx software version 4.1. Integration and quantification were performed using Waters Target Links™ software. For every dosage, calibration curves were determined with a mixture of ceramide containing various fatty acid chain length (C14:0 Cer, C16:0 Cer, C18:0 Cer, C18:1 Cer, C20:0 Cer, C24:0 Cer, and C24:1 Cer). A linear relationship between analyte response and concentration has been demonstrated by the linear standard curves obtained in the tested calibration range (0.15–25 pmol) for all ceramide compound ($r > 0.98$). The reproducibility of the plasma ceramide quantification was controlled by performing the average of three extractions with two LC–MS/MS injections for each sample. Coefficients of variation (CVs) were less than 10% for each plasma ceramide compound. Furthermore, an internal standard (C17:0 ceramide) was included in each sample (calibration point and plasma) and subjected to extraction and purification step, allowing a better quantification and the comparison between every sample.

Statistical analysis

Three replicate Cer measurements were performed on each plasma sample. Comparisons were made using Wilcoxon's signed-rank test and ANOVA with 95% confidence intervals (StatView 6.0). Cer variations between D3 or D10 and D0 for every patient were compared to the expression profiling of other patients by hierarchical clustering using Cluster [16] and Java TreeView softwares (http://bonsai.hgc.jp/~mdehoon/software/cluster/). Independence of groups was established by a Chi-squared test. Probability curves for tumour control were obtained by the Kaplan–Meier [17] and compared by the log–rank test. Hazard ratio (HR) of tumour control was assessed for groups with increased Cer vs. decreased Cer at D3 and 10. Prognostic value of variables (sex, age, tumour location and volume) was assessed using Cox's multivariate regression model [18]. *P* values smaller than 0.05 were considered statistically significant.

Results

Thirty-five patients (mean age, 64 years; range, 32–84; Table 1) met the inclusion criteria and received the complete treatment. There were 10 CR, 8 PR, 8 SD, and 9 PD.

Correlation between variations in total plasma Cer and tumour response

At D3, total plasma Cer was significantly increased over basal Cer level (D0) in patients with CR or PR (CR: 18.5% ± 8.92, $P < 0.05$; PR: 10.7% ± 2.27; $P < 0.01$), remained unchanged in patients with SD (−1.70% ± 4.36, $P > 0.05$), and fell significantly in patients with PD (−19.06% ± 5.72; $P < 0.05$) (Fig. 1A). At D10, Cer remained elevated in patients with CR or PR, but decreased significantly in patients with SD (−15% ± 2.40; $P < 0.01$) to reach a level approaching that of patients with PD (−20.16% ± 3.71; $P < 0.01$). The observed increases in total Cer level were not related to any covariate (patient sex and age, tumour location and volume; data not shown). Responders were defined either as CR + PR + SD or as objective responders (patients with tumour shrinkage, i.e. CR + PR), with both definitions yielding similar results. According to the first definition, total Cer was significantly higher than the basal level at D3 (9.91% ± 3.99; $P < 0.05$), but not at D10 (2.41% ± 4.6; $P > 0.1$; Fig. 1B). In refractory patients (defined as PD), total Cer was significantly decreased on both of these days (D3: −19.06% ± 5.72, $P < 0.05$; D10: −20.17% ± 3.71, $P < 0.01$). According to the second definition, total Cer level was also significantly higher than the basal level at D3 in objective responders (15.07% ± 5.02; $P < 0.01$) but not at D10 (10.35% ± 5.65; $P > 0.5$; Fig. 1C). It was significantly lower in non-responders (SD + PD) both at D3 (−9.79% ± 4.12, $P < 0.05$) and D10 (−17.95% ± 2.27, $P < 0.01$). At the difference to the follow-up during the treatment, basal levels of total plasma Cer were unable to discriminate between responders and non-responders (objective responders: 3.34 μM ± 0.32, non-responders: 3.82 μM ± 0.45; $P = 0.67$; Fig. 3A). Finally, growth of new metastases at distance to the irradiation location was similar independently of the tumour response of the patient (4/10 CR; 3/8 PR; 3/8 S; 4/9 PD) and was not correlated to Cer modulation at D3 or D10 (respectively, $P = 0.5$ and 0.79, Fig. S2B).

Correlation between variations in Cer subspecies and tumour response

Among the 12 investigated Cer subspecies, the most abundant were the 4 containing fatty acids C24:0 (44.39% ± 6.91), C24:1 (23.74% ± 6.43), C22:0 (16.06% ± 2.27), and C16:0 (7.51% ± 2.58). Three others (C18:0, C20:0, C22:1) were present in very small amounts (respectively 2.11% ± 0.86, 3.09% ± 1.91, 3.06% ± 1.99). The remaining 5 subspecies were undetectable. The patterns describing the changes in the levels of the 4 major Cer subspecies at D3 and D10 were very close to each other and to total Cer (Fig. 2A–D). For the most abundant subspecies (C24:0), the percentage increase over the basal value in objective responders was 11.9% ± 5.17 ($P < 0.05$) at D3. The percentage decrease in non-responders was −11.08% ± 4.33 ($P < 0.05$) at D3 and −19.19% ± 2.39 ($P < 0.05$) at D10 (Fig. 2C).

Hierarchization clustering of patients according to total plasma Cer

Clustering of patients according to the percentage change in total Cer level at D3 and D10 is shown in Fig. 3. Objective responders (8/10 CR and 6/8 PR) tended to be clustered above the median value ($P < 0.01$) and non-responders (5/8 SD and 8/9 PD) below the median value ($P < 0.01$). Measurement of changes in the levels of the 4 main Cer subspecies did not improve discrimination (data not shown).

Relationship between variations in Cer levels and tumour control at one year

Tumour volume was measured at 3, 6 and 12 months post treatment. Kaplan–Meier curves revealed that the probability of tumour control during the first year was high in patients with increased total plasma Cer levels. Estimation of the treatment efficacy was better at D10 with HR = 0 (95% CI: 0.044–0.58, $P < 0.01$), than at D3 with HR = 0.18 (95% CI: 0.048–0.56, $P < 0.01$; Fig. 4A and B). No patient with increased Cer at D10 showed a tumour volume development during the first year after treatment. Conversely, patients with decreased Cer have a 50% likelihood of showing a volume increase, and this risk is similarly at D3 with HR = 5.56 (95% CI: 1.76–21.71, $P < 0.01$) and D10 with HR = 6.21 (95% CI: 1.57–24.53, $P < 0.01$).

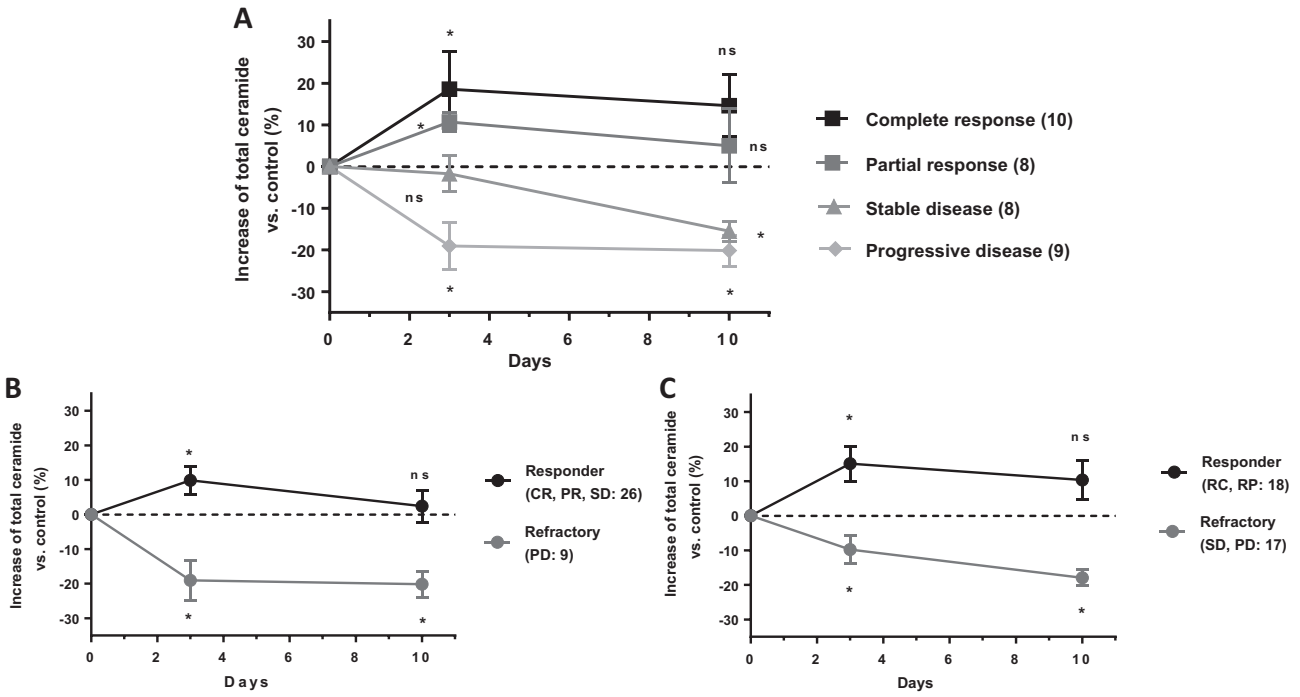

Fig. 1. Variations in total plasma Cer according to tumour response. Percentage change in Cer level at D3 and D10 (A) in patients with a complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD), (B) in responders (CR + PR + SD) versus refractory patients (PD), (C) in objective responders (CR + PR) versus non-responders (SD + PD). (Triplicate measurements, number of patients in parentheses, mean ± SEM, ns = $P > 0.05$, * = $P < 0.01$).

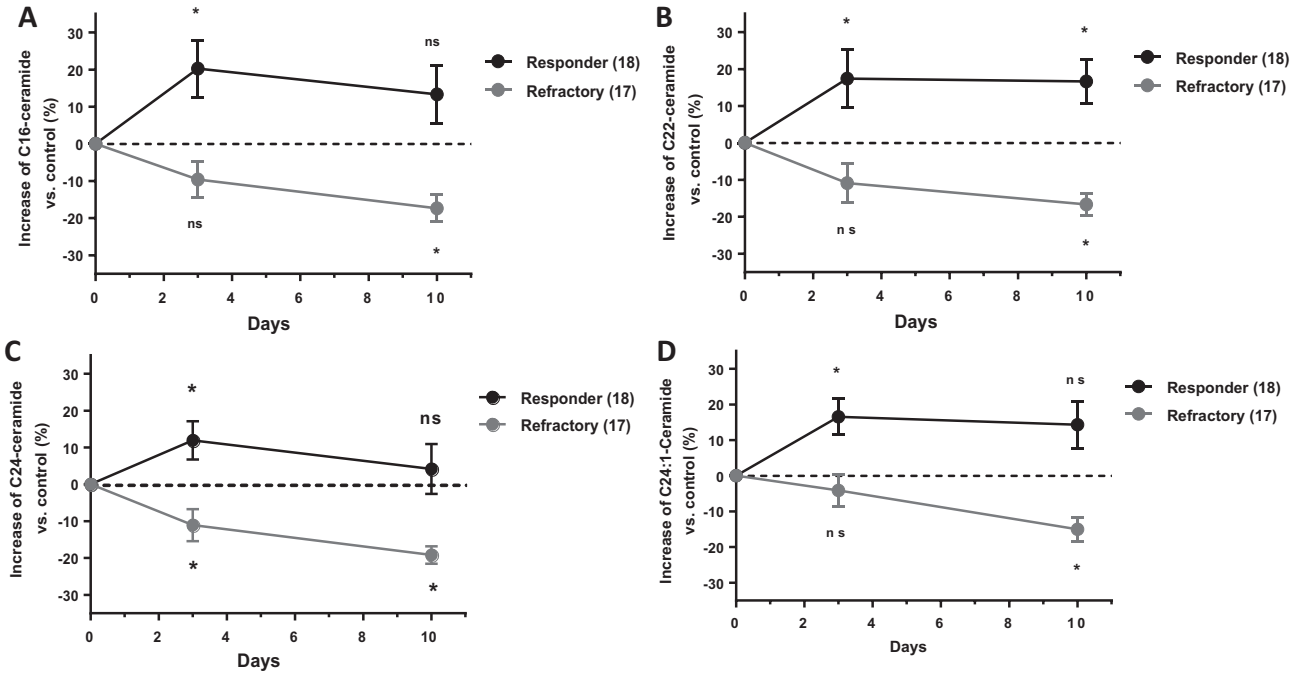

Fig. 2. Variations in the 4 main Cer subspecies according to tumour response. Percentage change in subspecies Cer levels at D3 and D10 (C16:0 (A), C22:0 (B), C24:0 (C) and C24:1 (D)), in objective responders (CR + PR) versus non-responders (SD + PD). (Triplicate measurements, number of patients in parentheses, mean ± SEM, ns = $P > 0.05$, * = $P < 0.05$).

Discussion

In our ancillary study of a phase II trial of SBRT testing with irinotecan against liver or lung metastases from colorectal cancer, plasma Cer levels were higher in patients than in healthy donors (data not shown), in line with published data on changes in Cer levels in a variety of diseases [8–11]. Total Cer levels before therapy were not indicative of tumour response. However, a significant increase in plasma Cer was recorded in objective responders unlike in non-responders early during the course of treatment. There was a correlation between elevated Cer levels at D3 or D10 of treatment and tumour response and between reduced Cer levels and stable or

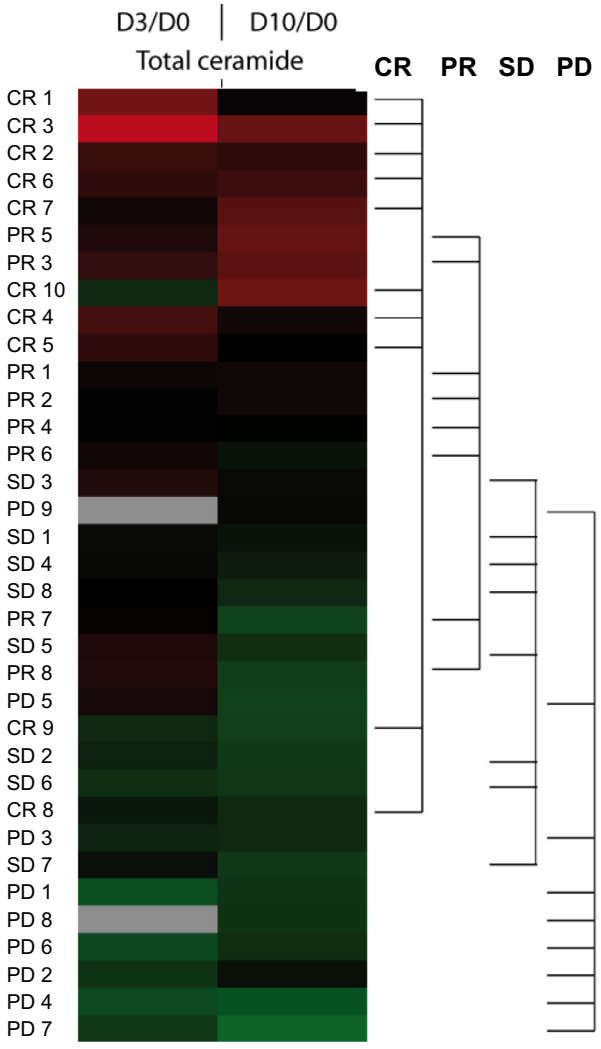

Fig. 3. Hierarchization clustering of patients according to total plasma Cer at D3 and D10. The position of each patient in the hierarchical tree for each response category (CR, PR, SD, PD) was obtained by Cluster 3.0 analysis and TreeView visualization. (Cer increase in red, Cer decrease in green, median 0 value in black).

progressive disease. This was confirmed in a cluster analysis of patients based on Cer levels which clearly discriminated between responders and non-responders. No variate (patient sex or age, tumour location or volume, number of metastases outside of irradiation field) correlated with elevated plasma Cer during treatment (data not shown). In Kaplan–Meier analyses, tumour control was function of plasma Cer elevation or reduction, with better estimates obtained for measurements made at D10 than D3. One year after treatment, there was any increase in tumour volume in patients with elevated Cer at D10 whereas 50% of patients with decreased Cer at D10 experienced an increase in tumour volume. Changes in total plasma Cer levels may thus constitute an early biomarker of tumour response to SBRT combined with irinotecan.

RECIST 1.1 has become a standard methodology for assessing definitive tumour response following radiotherapy [13]. However, accuracy has been challenged to detect lung tumour recurrence after hypofractionned radiotherapy. Target lesion may actually present fibrosis, and response might be miscategorized [19], so that tumour efficacy of SBRT with irinotecan is underestimated. However, the size of mass-like consolidation observed for lung tumours varied within 12 months after SBRT [20]. After 12 months, the size did not increase in all of the radiation-induced local injury cases, and has already increased in all recurrence cases. Finally, no quantitative method using only CT-scan permitted a robust and definitive estimation of the tumour volume. Again, future studies must include multimodal imaging, including PET-Scan and RMI. In our study, we may underestimate the efficacy of the SBRT with irinotecan, when tumour is showing stabilized volume one year after treatment. In SD group, some of the 8 patients may be responders, but the fibrosis on the tumour location does not permit to observe tumour regression. However, all SD are showing a slight decrease of ceramide and are ranked for high risk of tumour escape. Limit of RECIST 1.1 is comforting this statement. Those patients must be tightly followed-up with novel imaging modalities.

Our results expand on earlier observations in a small study demonstrating a significant increase in plasma Cer levels 72 h after SFGRT (1×15 Gy followed by 30×2 Gy) in 3/3 CR and 2/4 PR patients but detecting no correlation in non-responders (one increase and one decrease in level) [12]. The study was inconclusive because of the small number of patients ($n = 11$), and the diversity of tumours. Our study in 35 patients (18 responders and 17 non-responders) included only patients with metastases derived from primary colorectal carcinomas, receiving the same treatment and presenting tumours with a well-defined volume (largest diameter <6 cm), thereby limiting bias due to tumour volume.

In our study, not only total plasma Cer, but the 4 main Cer subspecies (C16:0, C18:0, C20:0, C22:0, C22:1, C24:0, and C24:1) were increased in objective responders. In non-responders, there was a decrease in Cer subspecies that was more manifested at D10 than D3. The pattern of changes in all Cer subspecies mirrored that in total Cer, implying that quantification of individual Cer subspecies would not improve the strength of total Cer as a biomarker.

In preliminary experimental work without added irinotecan, tumour irradiation in mice induced plasma Cer in a dose-dependent manner (unpublished data). The mechanisms underlying elevated plasma Cer levels are unclear, but we can offer at least two possible explanations. First, ASM and Cer are secreted into the extracellular medium by endothelial cells activated by pro-inflammatory cytokines including interleukin-β and tumour necrosis factor-α [21]. We observed such secretion after irradiation of primary microvascular endothelial cells HMVEC-L (unpublished data). According to this hypothesis, Cer release from endothelial activation would induce Cer-dependent radiosensitization of tumour cells. Secondly, the elevation in Cer might also be explained by induction by SBRT of a high level of tumour apoptotic bodies enriched in Cer. An unregulated increase in Cer might occur during the late non-reversible stage of cell death induced by DNA damage [22] and be a marker of radiation-induced cell death.

Use of plasma Cer as an early biomarker of tumour response may be a function of radiotherapy protocol, and elevated Cer may only be observable and quantifiable after high dose radiation. Commonly, radiotherapy protocols, use a fractionated dosing schedule of 2 Gy daily over several weeks. However, this dose may not be efficient to induce Cer within irradiated cells. Moreover, if Cer generation is slow, the early increase over baseline may not be significant. The newer radiotherapy modalities (e.g. stereotactic radiotherapy, intraoperative radiotherapy, protontherapy) that enable improved tumour targeting, dose escalation and use of fewer fractions might induce higher acute intracellular Cer levels and/or tumour cell death. Further investigations should demonstrate if plasma Cer is a large-range biomarker for innovative high-dose, but also conventional radiotherapies.

Plasma Cer need other complementary studies before being fully validated as a large broad biomarker of the radiotherapy efficacy. In this present manuscript, Cer analysis has been made on well-defined patients with lung or liver oligometastases from pri-

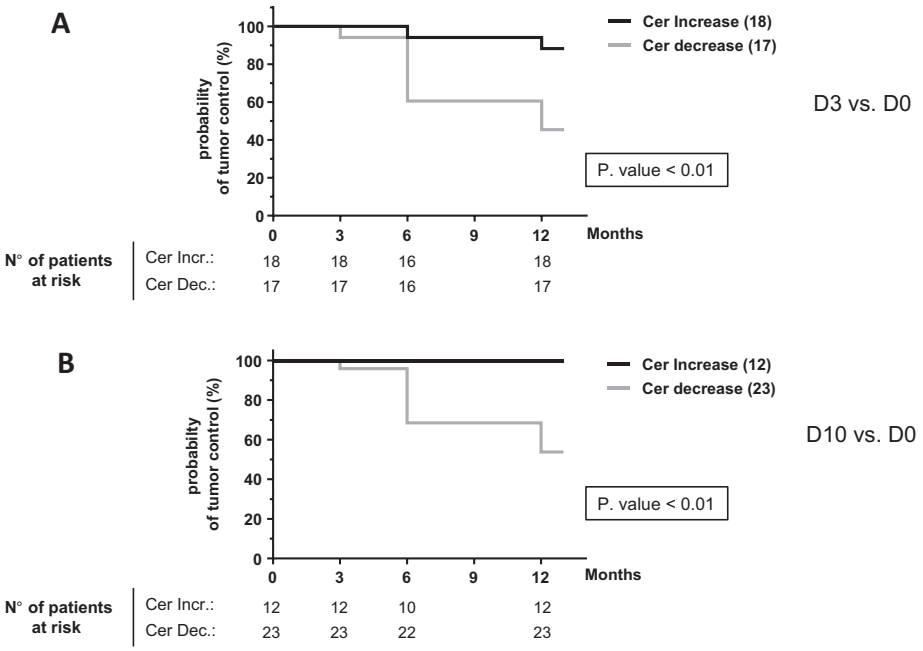

Fig. 4. Kaplan–Meier curves for tumour control over 1 year according to whether an increase or decrease in total plasma Cer at D3 (A) and D10 (B). (Number of patients in parentheses, increase vs decrease $P \leqslant 0.01$ at both D3 and D10). Inserted tables below the graphs indicate the number of patients evaluated by CT-scan for tumour control at every time point. 3 patients have not been evaluated at 6 months.

mary colon tumours to limit a potential heterogeneity of the response due to tumour localization or type. Future investigations must be performed on patients carrying on different tumour types treated by SBRT protocols. Another limit of our present study is due to the unclear impact of irinotecan on plasma Cer levels. Ongoing studies on cultured cells and mice are demonstrating that irradiation alone enhances Cer level in cell medium and plasma. Definitive answer will be provided by starting clinical studies on lung and liver tumours treated by SBRT without chemotherapy. In conclusion, the efficacy of radiotherapy protocols may not be judged until months after treatment. During this time, refractory patients may suffer from the complications of high-dose radiotherapy protocols providing no clinical benefit, do not have access to alternative treatments, and may experience tumour progression. Elevated plasma Cer levels early during the course of SBRT combined with irinotecan were found to be correlated with tumour response and reduced levels with a lack of response, making plasma Cer a potentially useful biomarker. The use of this biomarker should help physicians in the capacity to define patients with high risk of tumour escape to the treatment. Those patients will need a prompt and systematic post-treatment follow-up by multimodal imaging, such as MRI or F18-FDG PET scan. Moreover, the use of plasma Cer as a biomarker has the considerable practical advantage that plasma samples can be readily obtained throughout treatment whereas tumour biomarkers used in genomic and proteomic assays require tumour biopsies, which are much more costly and difficult to access especially if multiple biopsies are required [23].

Grant support

The work was funded by the Institut National Du Cancer (INCA), la Ligue Nationale Contre le Cancer and Electricité de France (EDF).

Conflict of interest

No conflict of interest.

Acknowledgements

We thank Audrey Bihoué and Valérie Pacteau for technical assistance and for clinical data management. We thank Dr. Julie Saba for critically reviewing the manuscript. Irinotecan (Campto®) was supplied by Pfizer laboratories.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.radonc.2016.03.014.

References

[1] Bernier J, Hall EJ, Giaccia A. Radiation oncology: a century of achievements. Nat Rev Cancer 2004;4:737–47.
[2] Kavanagh BD, Miften M, Rabinovitch RA. Advances in treatment techniques: stereotactic body radiation therapy and the spread of hypofractionation. Cancer J 2011;17:177–81.
[3] Bibault JE, Ceugnart L, Prevost B, Mirabel X, Lartigau E. CT appearance of pulmonary carcinomas after stereotactic radiation therapy. Diagn Interv Imaging 2013;94:255–62.
[4] Corre I, Niaudet C, Paris F. Plasma membrane signaling induced by ionizing radiation. Mutat Res 2010;704:61–7.
[5] Kimura K, Markowski M, Edsall LC, Spiegel S, Gelmann EP. Role of ceramide in mediating apoptosis of irradiated LNCaP prostate cancer cells. Cell Death Differ 2003;10:240–8.
[6] Rodriguez-Lafrasse C, Alphonse G, Aloy MT, Ardail D, Gerard JP, Louisot P, et al. Increasing endogenous ceramide using inhibitors of sphingolipid metabolism maximizes ionizing radiation-induced mitochondrial injury and apoptotic cell killing. Int J Cancer 2002;101:589–98.
[7] Garcia-Barros M, Paris F, Cordon-Cardo C, Lyden D, Rafii S, Haimovitz-Friedman A, et al. Tumor response to radiotherapy regulated by endothelial cell apoptosis. Science 2003;300:1155–9.
[8] Petrache I, Natarajan V, Zhen L, Medler TR, Richter AT, Cho C, et al. Ceramide upregulation causes pulmonary cell apoptosis and emphysema-like disease in mice. Nat Med 2005;11:491–8.
[9] Lang PA, Schenck M, Nicolay JP, Becker JU, Kempe DS, Lupescu A, et al. Liver cell death and anemia in Wilson disease involve acid sphingomyelinase and ceramide. Nat Med 2007;13:164–70.
[10] Delogu G, Famularo G, Amati F, Signore L, Antonucci A, Trinchieri V, et al. Ceramide concentrations in septic patients: a possible marker of multiple organ dysfunction syndrome. Crit Care Med 1999;27:2413–7.

[11] Watt MJ, Barnett AC, Bruce CR, Schenk S, Horowitz JF, Hoy AJ. Regulation of plasma ceramide levels with fatty acid oversupply: evidence that the liver detects and secretes de novo synthesised ceramide. Diabetologia 2012;55:2741–6.
[12] Sathishkumar S, Boyanovsky B, Karakashian AA, Rozenova K, Giltiay NV, Kudrimoti M, et al. Elevated sphingomyelinase activity and ceramide concentration in serum of patients undergoing high dose spatially fractionated radiation treatment: implications for endothelial apoptosis. Cancer Biol Ther 2005;4:979–86.
[13] Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228–47.
[14] Hara A, Radin NS. Lipid extraction of tissues with a low-toxicity solvent. Anal Biochem 1978;90:420–6.
[15] Bodennec J, Koul O, Aguado I, Brichon G, Zwingelstein G, Portoukalian J. A procedure for fractionation of sphingolipid classes by solid-phase extraction on aminopropyl cartridges. J Lipid Res 2000;41:1524–31.
[16] Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci USA 1998;95:14863–8.
[17] Kaplan P. Non parametric estimation from incomplete observation. J Am Stat Assoc 1958;53:457–80.
[18] Cox D. Regression models and life tables. J R Stat Soc 1972;34:187–220.
[19] Mattonen SA, Huang K, Ward AD, Senan S, Palma DA. New techniques for assessing response after hypofractionated radiotherapy for lung cancer. J Thorac Dis 2014;6:375–86.
[20] Matsuo Y, Nagata Y, Mizowaki T, Takayama K, Sakamoto T, Sakamoto M, et al. Evaluation of mass-like consolidation after stereotactic body radiation therapy for lung tumors. Int J Clin Oncol 2007;12:356–62.
[21] Marathe S, Schissel SL, Yellin MJ, Beatini N, Mintzer R, Williams KJ, et al. Human vascular endothelial cells are a rich and regulatable source of secretory sphingomyelinase. Implications for early atherogenesis and ceramide-mediated cell signaling. J Biol Chem 1998;273:4081–8.
[22] Tepper AD, de Vries E, van Blitterswijk WJ, Borst J. Ordering of ceramide formation, caspase activation, and mitochondrial changes during CD95- and DNA damage-induced apoptosis. J Clin Investig 1999;103:971–8.
[23] Ganepola GA, Nizin J, Rutledge JR, Chang DH. Use of blood-based biomarkers for early diagnosis and surveillance of colorectal cancer. World J Gastrointest Oncol 2014;6:83–97.