

Qualification of a Noninvasive Magnetic Resonance Imaging Biomarker to Assess Tumor Oxygenation

Florence Colliez, Marie-Aline Neveu, Julie Magat, Thanh Trang Cao Pham, Bernard Gallez, and
Bénédicte F. Jordan

Abstract

Purpose: Although hypoxia has been long recognized as a crucial factor impairing tumor response in many therapeutic schemes, atraumatic and reliable methods of individually quantifying tumor oxygenation are still lacking in day-to-day clinical practice. The aim of this work was to investigate the potentially quantitative properties of our recently described noninvasive magnetic resonance (MR) technique "MOBILE" (mapping of oxygen by imaging lipids relaxation enhancement) and to qualify this endogenous contrast as a tumor hypoxia marker.

Experimental Design: The "MOBILE" technique, which assesses the longitudinal MR relaxation rate, R_1 , of lipid protons, was benchmarked with the parent technique which assesses the global (or water) R_1 , in response to a hyperoxic challenge (carbogen breathing) and to a hypoxic challenge (combretastatin A4) in MDA-MB-231 xenografts and in NT2 mammary tumors. Electron paramagnetic resonance oximetry was used to quantitatively assess the tumor pO_2 in matching tumors longitudinally.

Results and Conclusion: Our study evidenced that (i) positive and negative changes in tumor oxygenation can be detected using MOBILE; (ii) a change in the R_1 of lipids is positively correlated with a change in the tumor pO_2 ($P = 0.0217$, $r = 0.5097$); (iii) measured lipid R_1 values are positively correlated with absolute pO_2 values in both tumor models ($P = 0.0275$, $r = 0.3726$); and (iv) changes in the R_1 of lipids are more sensitive than changes in the global R_1 . As this technique presents unique translational properties, it seems promising for the individual longitudinal monitoring of tumor oxygenation in a clinical setting.

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Introduction

Tumor hemodynamics has become a key target in pre-clinical and translational cancer research (1), involving both negative and positive modulations of tumor oxygenation/perfusion. On the one hand, attempts are made to target the established tumor vasculature or neovasculature by the use of antivascular or antiangiogenic agents (2–6), whose effects are traditionally assessed noninvasively not only using dynamic contrast MRI (DCE-MRI) after administration of an exogenous paramagnetic contrast agent (7–9), but also possibly using endogenous hemodynamic markers, including BOLD-MRI (blood oxygen level dependent MRI; ref. 10). On the other hand, positive modulations of tumor hemodynamics, tumor hypoxia, or environmental pH, for example, (11), are being considered in the field of tumor radiosensitization (12–14).

Most solid tumors contain regions of acute and chronic hypoxia that indicate a negative clinical outcome after radiotherapy (15). To bridge the gap between the occurrence of tumor hypoxia and clinical radiation practice, there is an essential need to predict the presence of hypoxic regions in tumors individually. On the basis of the individual tumor characteristics and/or the ability to alleviate the tumor hypoxia, it will become possible to adapt the individual treatment either by boosting optimal radiation doses in the resistant areas, by adapting the radiotherapy to each tumor throughout the course of treatment, or by administering an associated treatment to potentiate the effectiveness of the radiation treatment.

There is therefore a critical need to develop accurate, noninvasive and quantitative *in vivo* imaging methods of mapping tumor oxygenation in cancer management, both for the purpose of targeting tumor blood vessels and for the oxygen-induced resistance to radiation of tumors. Noninvasive, safe, and repeatable techniques to map tumor hypoxia are therefore required.

Direct quantitative methods, including Eppendorf microelectrodes (16), electron paramagnetic resonance (EPR) oximetry (17), ^{19}F relaxometry (18), or Overhauser enhanced MRI (19), are either invasive or require the injection of a reporter probe, and are currently not clinically

Biomedical Magnetic Resonance Group, Louvain Drug Research Institute, Université Catholique de Louvain, Brussels, Belgium

Corresponding Author: Bénédicte Jordan, UCL, Louvain Drug Research Institute, Avenue Mounier 73, Box B1.73.08, B-1200 Brussels, Belgium. Phone: 3227647364; Fax: 3227647390; E-mail: benedicte.jordan@uclouvain.be

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Translational Relevance

This study describes MOBILE (mapping of oxygen by imaging lipids relaxation enhancement) as a sensitive, noninvasive endogenous marker of tumor oxygenation, as the technique is sensitive to both positive and negative modulations of tumor oxygenation and, more importantly, significantly correlated to actual pO_2 values in matching tumors for both tumor models being studied. The MOBILE technique presents unique translational properties and seems promising for further clinical monitoring of individual tumor oxygenation with potential applications for the planning of radiotherapy or for assessing the response to anti-angiogenic or anti-vascular treatments.

applicable. One technique for mapping hypoxia in the current clinical setting is PET using nitroimidazole-derived tracers accumulating within hypoxic areas (20, 21). However, cost, reduced availability, and radiation exposure preclude its use in routine longitudinal practice. Moreover, it does not allow the monitoring of acute changes in hypoxia because it requires an accumulation of the tracer in the tissue of interest for several hours, and does not allow the follow-up of dynamic changes induced by oxygenation modulators. Ideally, imaging hypoxia in humans should be noninvasive, sensitive, robust, quantitative, widely available, and able to probe tumor heterogeneity. To this end, two endogenous sources of contrast in MRI, namely functional MRI and oxygen-enhanced MRI, are currently being explored (22). T_2^* (effective transversal relaxation rate) mapping, also referred to as functional MRI or "BOLD" imaging (23), is sensitive to changes in oxygenation in the vascular compartment and has demonstrated significant limitations in terms of quantitative relationships between response signal intensity and true changes in tumor tissue pO_2 (24–26). Moreover, it is also sensitive to changes in the basal hemoglobin content. More recently, changes in tissue oxygen concentrations have been shown to produce changes in the relaxation rate R_1 ($= 1/T_1$) of water ("oxygen enhanced MRI"; refs. 27, 28, 29). T_1 demonstrates sensitivity to dissolved oxygen, thereby acting as a potential T_1 -shortening paramagnetic contrast agent (19). An oxygen-induced increase in the R_1 has the potential to provide noninvasive measurements of fluctuations in the oxygen level of tissue, as a complement to BOLD imaging (30, 31). Unfortunately, this technique is still hampered by insufficient sensitivity (only a few percentage changes) and the ΔR_1 measured may be biased owing to confounders unrelated to changes in the oxygen level in the tissue, such as an alteration in blood flow, and the H_2O content of the tissue (32).

We recently described a new noninvasive MRI method allowing rapid mapping of changes in tissue oxygenation and based on the higher solubility of oxygen in lipids than in water. By monitoring changes in the R_1 relaxation rate of

the lipid peak rather than those in the water peak, sensitive estimates of variations in tissue oxygenation could be obtained (33, 34). This sequence, with the acronym MOBILE for "mapping of oxygen by imaging lipids relaxation enhancement," has simultaneously been translated into the clinical setting because it enables noninvasive and sensitive measurements of changes in tissue oxygenation. Until now, MOBILE has been successfully applied in hypoxic mouse models mimicking physiopathological conditions such as tumor hypoxia, peripheral ischemia, cerebral ischemic stroke, and liver steatosis, as well as in the clinical setting in healthy volunteers (33) and in stroke patients (35).

The aim of the current work was to investigate the potentially quantitative aspect of MOBILE, as a biomarker for tumor oxygenation in two mammary tumor models (NT2 and MDA-MB-231), to benchmark MOBILE in comparison with the more traditional endogenous contrast using the global R_1 , and to assess the ability of the technique to monitor positive and negative changes in tumor oxygenation. Hypoxic challenges were performed by the injection of the antivascular agent CA4, described to induce a vascular shutdown as early as 3 hours after the administration of the drug (36), whereas hypoxic challenges were induced by a carbogen breathing (95% O_2 /5% CO_2) known to acutely increase tumor oxygenation in a wide range of tumor models/xenografts and in human tumors (36–40, 31). Oxygenation was assessed in matching tumors (the same tumors underwent both EPR and MRI experiments) using EPR oximetry as a method of reference for the quantitative assessment of the tumor pO_2 .

To this end, the following questions will be addressed: "Is a change in the R_1 (ΔR_1) of lipids related to a change in the tumor pO_2 (ΔpO_2)?"; "Are intrinsic R_1 values of lipids related to absolute pO_2 values?"; and finally, "Are changes in the R_1 of lipids more sensitive than changes in the global R_1 ?"

Materials and Methods

Tumor models

A total of 7×10^6 NT2 cells (provided by E. Jaffee) or 10×10^6 MDA-MB-231 (LGC Promochem), amplified *in vitro*, were collected by trypsinization, washed three times with Hanks Balanced Salt Solution (HBSS), and resuspended in 100 μ L HBSS. These mammary tumor cells were injected subcutaneously into the right upper mammary fat pad of 6-week-old FVB/N or nude NMRI female mice (Janvier). The tumors were analyzed when they reached 5 mm in diameter. The animals were anesthetized by inhaling isoflurane (Forene) mixed with 21% oxygen (air) in a continuous flow (1.5 L/hour). This anesthetic has been shown not to interfere with tissue hemodynamics (41). The respiration rate and body temperature ($37.0^\circ\text{C} \pm 1.0^\circ\text{C}$) were monitored and maintained with a circulating water blanket. Studies were undertaken in accordance with the national and local regulations of the ethical committee (agreement number UCL/2010/MD/001).

Hypoxic and hyperoxic challenges

To negatively modulate tumor oxygenation, CA4 was administered at a dose of 100 mg/kg and MR measurements were taken before injection and repeated 3 hours after the administration of CA4. EPR oximetry was performed on the same tumors, in a similar way, before injection and directly after the second MRI session. To positively modulate tumor oxygenation, carbogen breathing (95% O₂, 5% CO₂) was used. For that purpose, three MR measurements of each type (global R₁ and R₁ of lipids) were taken sequentially and repeated three times while the subject was breathing air. The gas was then switched to carbogen, and MR measurements were repeated at 10, 15, and 20 minutes after the switch, as it is known that oxygenation is significantly increased after 10 minutes' breathing (42). EPR experiments were subsequently performed on the same tumors. We considered five hyperoxic challenges and five hypoxic challenges in each tumor model. Both challenges could have been applied on the same tumor for three NT2 tumors and two MDA-MB-231 tumors. The remaining experiment was performed on different tumors.

MR experiments

T₁ measurements.

A segmented IR FISP (Inversion-Recovery Fast Imaging with Steady state Precession) sequence (SSFP FID mode) was used to acquire parametric images of T₁ relaxation time, as described previously (33). Briefly, the acquisition parameters were TR/TE/FA/BW/matrix = 4 ms/1.2 ms/5°/100 kHz/64 × 64, four segments, and a total acquisition time of 1 minute 20 seconds. For the global proton experiment (global R₁, essentially reflecting the water peak), a series of 100 images were taken, with a slice thickness of 1 mm. For the lipid experiments (MOBILE), the offset between water and lipid peaks was assessed experimentally with a single pulse sequence (the lipid peak of interest was ~4.0 ppm) and then used as an imaging frequency offset in the same IR FISP protocol. We added a saturation pulse to spoil the water signal. A series of 40 images (spaced by scan repetition time, TR = 100 ms) with a slice thickness of 2 mm were acquired with a spatial resolution of 0.344 × 0.344 mm. The images were then fitted using a home-made program written in Matlab (The MathWorks, Inc.) to determine the T₁ relaxation time in the regions of interest (ROI), as described previously (33). **EPR oximetry.**

The *in vivo* tumor pO₂ was monitored using EPR oximetry, with charcoal as the oxygen-sensitive probe (17). EPR spectra were recorded using a 1.1 GHz EPR spectrometer (Magnetech). Calibrations curves were made by measuring the EPR line width as a function of the pO₂. Mice were injected in the center of the tumor using the suspension of charcoal (100 mg/mL, 40 µL injected) 24 hours before the experiment. The tumor being studied was placed in the center of the extended loop resonator whose sensitive volume extends 1 cm into the tumor mass. The pO₂ measurements correspond to an average of pO₂ values in the volume. MOBILE and EPR measurements were taken the same day as we know from unpublished data that a carbo-

gen challenge on one tumor is reproducible on a same day within a 3-hour interval.

Statistical analysis

ROIs were delineated by hand to select the tumor tissue without charcoal, although it has been previously shown that charcoal does not interfere with T₁ measurements (33). As we recorded three parametric maps of the global R₁, and three parametric maps of the R₁ of lipids, as well as three EPR measurements in each condition (at baseline, during carbogen breathing and 3 hours after CA4 administration), from each set of three measurements, we calculated the mean as well as the SD used for the correlations. Pearson correlations between the relaxation rates R₁ (or ΔR₁) and the actual pO₂ values (or ΔpO₂) were performed using the GraphPad software. Deming regression was performed to compare the sensitivities of the techniques.

Results

MOBILE enables the follow-up of positive and negative variations in tumor oxygenation

Tumor oxygenation was modulated in two distinct tumor models, one syngenic (NT2 mammary tumors) and one xenograft (MDA-MB-231 xenografts), using a hypoxic challenge (i.e., administration of CA4 3 hours before measurement) or a hyperoxic challenge (i.e., carbogen breathing). Two MDA-MB-231 tumors underwent both challenges, whereas six other tumors underwent hyperoxic (*n* = 3) or hypoxic challenges (*n* = 3) only. Three NT2 tumors were then submitted to both challenges and four other tumors were submitted to carbogen breathing (*n* = 2) or were targeted by CA4 (*n* = 2). Typical maps of the R₁ of lipids at baseline, and both after a hypoxic and a hyperoxic challenge are shown in Fig. 1, together with similar challenges assessed using global R₁ mapping.

A change in the R₁ of lipids is related to a change in the tumor pO₂

While pooling data from 20 different tumors (10 of each tumor model), we can observe a significant correlation between the ΔR₁ of lipids (MOBILE) and the ΔpO₂ in the same tumors (*P* = 0.0059, *r* = 0.5097; Fig. 2A), whereas similar analysis using the global R₁ does not give a significant correlation (Fig. 2B). Individual changes in the R₁ corresponding to an increase in absolute pO₂ values, as assessed by EPR oximetry, evidence that (i) the R₁ of lipids increased concomitantly (by 2%17% of relative increase) when the pO₂ increased, except for one tumor (2.7% decrease; Fig. 2A) and (ii) the global R₁ increased from 0% to 6% with respect to the same pO₂ increase, except for two tumors which showed decreases of 0.5% and 7% (Fig. 2B). As regards changes in the R₁ with respect to a decline in the pO₂, the graphs in Fig. 2 show that (i) the R₁ of lipids falls systematically (by 0.1%–13.6%; Fig. 2A) and (ii) the global R₁ shows mixed results with both negative (down 4.4%) and positive (up 5.6%) changes in matching tumors (Fig. 2B).

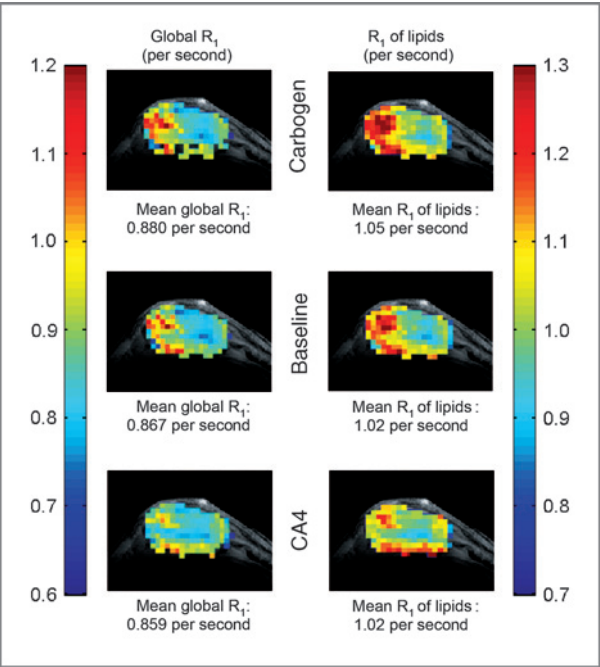


Figure 1. Typical maps of global R_1 and R_1 of lipids obtained on the same mice at baseline, and after hyperoxic and hypoxic challenges performed with carbogen and CA4, respectively. For this tumor, actual values of pO_2 was 6.1 mm Hg at baseline increased to 9.0 mm Hg during the carbogen breathing, and decreased to 5.1 mm Hg 3 hours after CA4 administration. Those maps illustrate the heterogeneity of response: it seems that the left part of the tumor is more influenced by oxygenation modulators (colors are warmer after the carbogen breathing and cooler after CA4 administration), whereas the right part is less sensitive to both agents. Moreover, intensity variations on the R_1 of lipids maps are higher than the changes observed with global R_1 .

Individual R_1 values of lipids are related to absolute pO_2 values

To investigate the potentially quantitative properties of MOBILE, mean individual R_1 values of lipids were com-

pared with individual pO_2 values within each tumor model. For this purpose, all pre- and postchallenge mean values of the R_1 of lipids (MOBILE) were plotted together with respect to all corresponding pre- and postchallenge pO_2 values (Fig. 3A and B).

Since some tumors underwent both challenges, only one basal value of each parameter (pO_2 , global R_1 and R_1 of lipids) was assessed for these tumors, resulting in a number of points that differ from 20: we considered 17 points and 18 points for the NT2 and MDA-MB-231 models, respectively. We were able to observe a positive correlation between the R_1 values of lipids and absolute pO_2 values in both models [$P < 0.0001$, $r = 0.8164$ and $P = 0.0378$, $r = 0.5069$] for MDA-MB-231 and NT2 tumor models, respectively], and between global R_1 and pO_2 values in the MDA-MB-231 tumor model ($P = 0.0025$, $r = 0.6673$).

When all the data were pooled, a significant correlation was established between the R_1 values of lipids and the pO_2 values ($P = 0.0275$, $r = 0.3726$; Fig. 4). We could not pool the global R_1 data from both tumor models because there is no correlation in the NT2 tumor model between pO_2 values and global R_1 values. All these data argue in favor of qualifying MOBILE as a sensitive method of assessing tumor oxygenation.

Furthermore, according to Fig. 4, the relaxation rates of lipids calculated within the MDA-MB-231 tumor model seem to be superior to those obtained in the NT2 tumor model. This observation goes hand in hand with the difference in oxygenation levels that was evidenced by EPR oximetry: MDA-MB-231 tumors are well oxygenated at baseline (10.5 ± 6.5 mm Hg) compared with NT2 tumors (4.8 ± 3.1 mm Hg).

Relative changes in the R_1 of lipids are more sensitive than relative changes in the global R_1

Sensitivities were assessed by comparing the slopes of the linear regression graphs for traditional oxygen-enhanced MRI and MOBILE data. This comparison can only be assessed within the MDA-MB-231 model because both

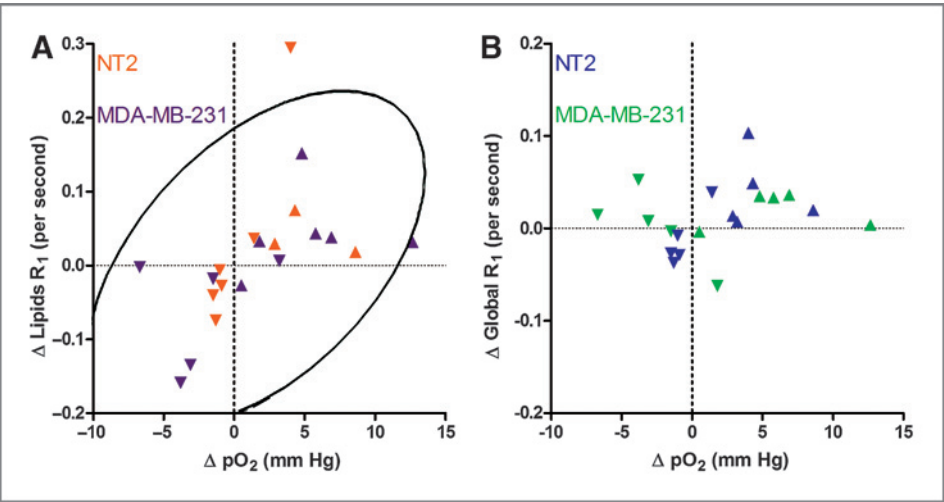
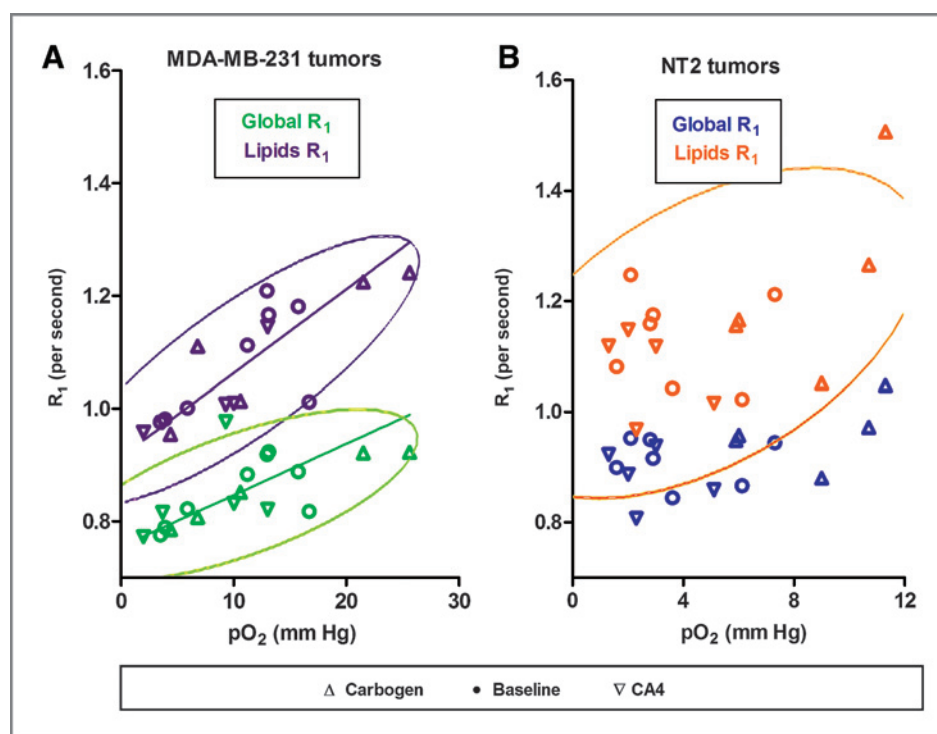


Figure 2. Variations of pO_2 , global R_1 , and R_1 of lipids were recorded during five hyperoxic challenges and five hypoxic challenges in each tumor model. Each triangle represents a variation of R_1 and the orientation of the symbol indicates whether it follows carbogen breathing (the symbol points up) or CA4 administration (the symbol points down). Variations of R_1 of lipids (provided by MOBILE) are significantly correlated to pO_2 changes (registered by EPR oximetry; A), whereas no significant correlation was established between pO_2 changes and global R_1 variations (B).

Figure 3. Mean values of R_1 of lipids and global R_1 (calculated from the three measurements performed in each condition) are compared with mean pO_2 values (also obtained at baseline, or after both challenges) within MDA-MB-231 tumors and NT2 tumor models in graphs A and B, respectively. A significant correlation is observed between R_1 of lipids values and pO_2 values in both models. On the contrary, a significant correlation could only have been established between global R_1 and pO_2 values in the MDA-MB-231 tumor model. Deming regression lines are added in the MDA-MB-231 model to compare the techniques sensitivities.



techniques show a significant correlation in this tumor model, which is not the case for the NT2 model. When comparing the slopes providing by the Deming regressions ($Y = 0.01486 X + 0.9143$ for Lipids R_1 and $Y = 0.01486 X +$

0.9143 for global R_1), we observe that MOBILE is approximately 1.5 times more sensitive than global R_1 measurement.

The magnitude of response to CA4 is related to basal pO_2

Figure 5 presents the magnitude of response in terms of the global R_1 , the R_1 of lipids, and the pO_2 , in relation to basal means of these three parameters. Although the response to a carbogen breathing challenge does not depend on basal pO_2 (Fig. 5A), as already published (42), the fall in pO_2 induced by CA4 is greater when the basal oxygenation level is higher. This is also assessed using the R_1 of lipids (Fig. 5C). However, neither basal values of the R_1 of lipids nor the global R_1 can help predict the extent of the response to carbogen or CA4 (Fig. 5D-I).

Discussion

This study demonstrates the ability of MOBILE to follow positive and negative changes in tumor oxygenation further to hypoxic or hyperoxic challenges, suggesting that the endogenous source of contrast relying on the R_1 of lipids in MRI can constitute a sensitive noninvasive marker of tumor hypoxia. The MOBILE technique enables the assessment of the relaxation parameter " R_1 of lipids" and is consecutive to a parent emerging technique assessing the "global R_1 ," which is prominently influenced by the R_1 of water, but lacks good sensitivity to changes in oxygenation. As R_1 techniques are sensitive to tissue oxygenation, they appear to be complementary to the routinely used

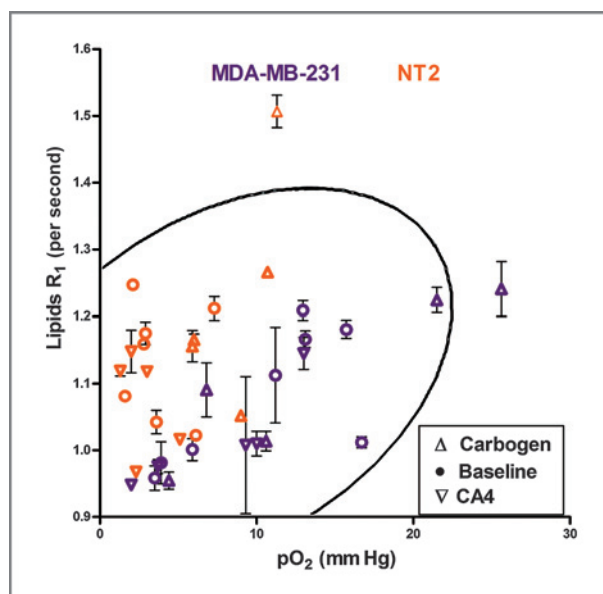


Figure 4. Mean relaxation rate values obtained from both tumor models are pooled on same graphic. Each point represents a mean R_1 of lipids value calculated from the three measurements at baseline (circle) and after the hypoxic or hyperoxic challenges (triangles pointing down or up, respectively) with its SD. A significant correlation is shown between R_1 of lipids and pO_2 values.

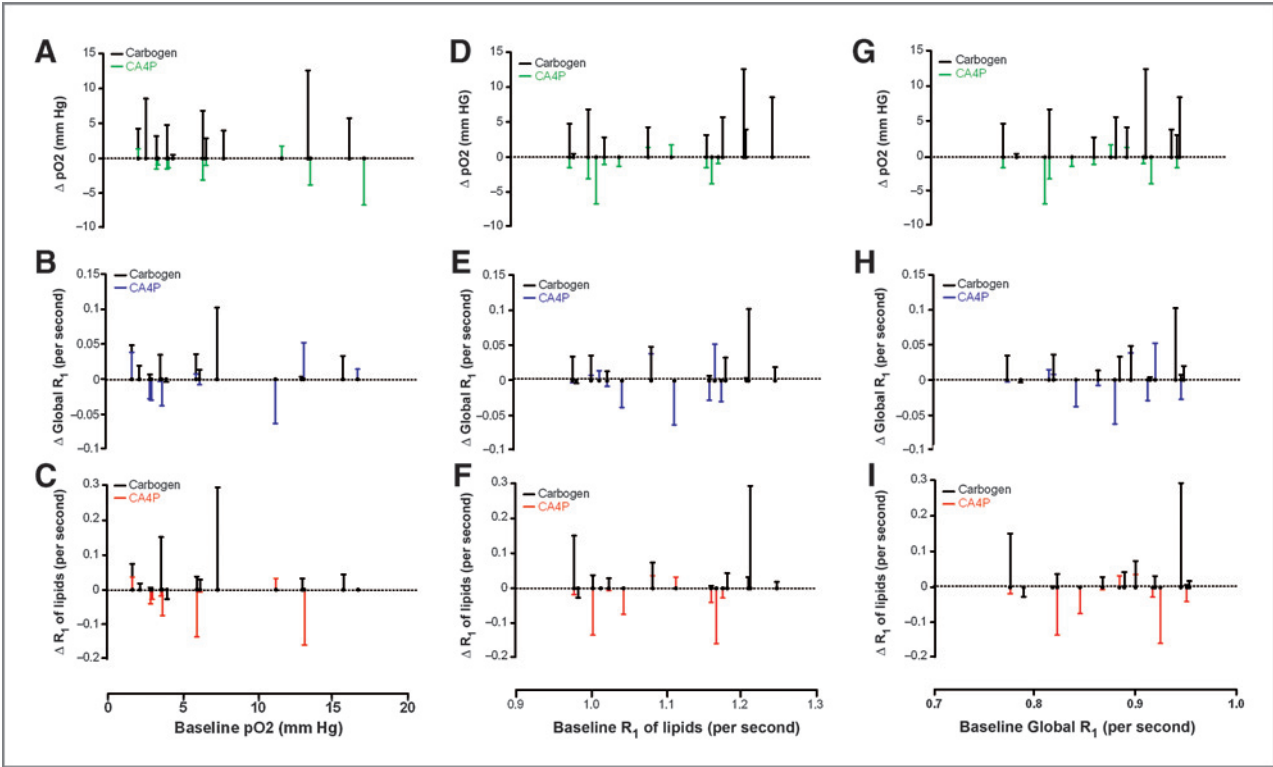


Figure 5. Black bars correspond to variations observed during a hyperoxic challenge, whereas colored bars correspond to variations in the parameters induced by the injection of CA4. A–C, modulations of the three parameters (pO_2 , global R_1 , and R_1 of lipids) are presented in function of actual pO_2 measured by EPR oximetry. It appears that amplitude of response to a carbogen breathing challenge is not dependent on the basal pO_2 . Contrarily, we can observe that the decrease in the R_1 of lipids and pO_2 is enhanced by a higher oxygenation level at baseline. D–F, changes of the three parameters in function of the mean R_1 of lipids value at baseline. From this graph, we cannot predict from a basal R_1 of lipids value whether the parameters will follow a small or a large variation after carbogen breathing or CA4 administration. **G and H**, variations of the three parameters are presented in function of mean global R_1 values at baseline. Here again, we cannot predict the magnitude of the response to hypoxic or hyperoxic challenges from the basal global R_1 value.



functional imaging or BOLD-MRI technique, assessing changes in the R_2^* relaxation parameter (29), which is sensitive to changes in oxygenation in the vascular compartment, yet with significant limitations in terms of quantitative aspects and in sensitivity.

Further validation of the hypoxia marker requires a correlation of the new method with a quantitative method in the preclinical setting. This was assessed using EPR oximetry, an invasive but quantitative and highly sensitive method able to assess tissue oxygenation *in vivo* (17). Our study evidenced that (i) positive and negative changes in tumor oxygenation can be detected using MOBILE; (ii) a ΔR_1 of lipids is positively correlated with a ΔpO_2 *in vivo*; (iii) individual R_1 values of lipids are positively correlated to absolute pO_2 values; and (iv) changes in the R_1 of lipids are more sensitive than changes in the global R_1 . This makes MOBILE a sensitive method to assess changes in tumor oxygenation. This is not systematically the case for the global R_1 , showing global R_1 changes that are not always correlated to the changes in the pO_2 and that are also smaller in magnitude (less sensitive), as observed on individual graphs.

The ability of the MOBILE technique to follow the effect of an antivascular agent (combretastatin A4) longitudinally

as well as that of a hyperoxic challenge (carbogen breathing) noninvasively could find direct applications in the clinical setting for the individual monitoring of patients treated using anticancer agents. To this end, individual monitoring of the actual effect of a drug on individual tumor hemodynamics could help the clinician in therapeutic decisions. This has also prompted efforts to combine antiangiogenic or antivascular agents together or with other treatment modalities (43–46).

When investigating the potentially quantitative properties of MOBILE (i.e., comparison of individual R_1 values of lipids vs. the actual pO_2 values), the R_1 values of lipids were shown to be correlated in individual models and on pooled data from both tumor models, whereas the global R_1 was not able to show such correlation. The significant correlations between R_1 of lipids and pO_2 argue in favor of a potential quantitative aspect of the MOBILE technique. However, although EPR oximetry measurements have shown that MDA-MB-231 tumors exhibit higher oxygenation level than NT2 tumors, the difference between the R_1 values of lipids at baseline between the two tumor models could imply that this parameter is also tissue dependent. Therefore, we cannot exclude that the R_1 is also influenced by tissue type and composition,

especially for tumor oxygenation lower than 5 mm Hg. It should be noted that tissue type dependency would also be applicable for the global R_1 . Moreover, on the single MDA-MB-231 tumor model, the global R_1 also correlated positively with the absolute pO_2 values. Accordingly, MOBILE seems to be more adapted to assessing tumor oxygenation than the parent technique assessing the global R_1 , in the tumor models included in this study, and is systematically more sensitive than the global R_1 . Nevertheless, both techniques remain complementary in nature because the origin of the information is different for lipid and global R_1 , and because it is not proven, yet that "MOBILE" could be applied on tumor models with a low content of lipids.

In the field of radiotherapy, individual monitoring and quantification of tumor oxygenation could find application in the identification of a therapeutic window during which oxygenation is effectively modified following treatment aimed at modifying oxygen supply and/or consumption. Overall modification of tumor hypoxia has been shown to significantly improve the efficiency of RT for locoregional control and for overall survival (47). Recent reports have also pointed out that the hypoxia-targeted approach ARCON (accelerated radiotherapy plus carbogen inhalation and nicotinamide) had an impact on the patients' outcome in hypoxic laryngeal tumors but not in well-oxygenated tumors (48), outlying that segmentation of the patients with respect to their basal oxygenation level is mandatory for the optimization of radiotherapy. The MOBILE technique could therefore help in the proper qualification of drugs targeting hypoxia. In addition, the technique could guide intensity modulated radiotherapy planning (IMRT; refs. 49–51) currently used in the clinical setting, which enables millimetric irradiation of each individual tumor to boost the radiation dose in the most hypoxic regions. Importantly, the time and spatial resolutions of the MOBILE technique are compatible with longitudinal monitoring of tumor oxygenation with sufficient spatial resolution to guide IMRT. IMRT is currently not guided in day-to-day clinical practice using hypoxia tracers (52), because none of them are as yet adapted for the purpose.

As far as we know, there is no direct, quantitative, dynamic, and accurate noninvasive/irradiating method that is part of routine clinical practice to assess tumor oxygenation. That is why, despite the dispersion of our data in correlation graphs, that prevents its use in routine clinical practice for exact pO_2 measurement at this stage, the significant correlation found between the R_1 of lipids and the pO_2 using MOBILE makes this a very interesting tool for assessing tumor oxygenation variations. This will need to be further evaluated in additional preclinical and

clinical studies. Experiments showing positive correlations in a wider range of tumor models and on other types of tissues, as well as a cross-validation with an alternative clinically available method able to map tumor hypoxia (i.e., PET imaging with nitroimidazoles), would be required.

In conclusion, the current study qualifies MOBILE as a sensitive, noninvasive, and potentially quantitative endogenous marker of tumor oxygenation, as the technique is sensitive to both positive and negative modulations of tumor oxygenation and, more importantly, significantly correlated to actual pO_2 values in matching tumors for both tumor models being studied. As the MOBILE technique presents unique translational properties and can be successfully implemented in the clinical setting, it is promising for further clinical monitoring of individual tumor oxygenation to assess the response to antiangiogenic or antivasculature treatments, for treatment combination and for planning radiotherapy. If further characterization is required for pure quantitative routine clinical applications, the technique can be considered to study modulations of tumor oxygenation in patients and clinical intratumoral heterogeneity.

Disclosure of Potential Conflicts of Interest

B.F. Jordan, J. Magat, and B. Gallez are co-inventors of a patent on *in vivo* quantification of a variation in a tissue by using a MRI technique. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: B. Gallez, B.F. Jordan
Development of methodology: J. Magat, B. Gallez
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): F. Colliez, M.-A. Neveu, T.T.C. Pham, B. Gallez
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): F. Colliez, B. Gallez
Writing, review, and/or revision of the manuscript: F. Colliez, B. Gallez, B.F. Jordan
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): F. Colliez, M.-A. Neveu
Study supervision: B. Gallez, B.F. Jordan

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Changes and corrections from the author:

Line 17: ' (EPR) ' is missing between resonance and oximetry
Line 97: Should we have '27-29' instead of '27,28,29' ?
Line 215: 'EPR oximetry' should go to next line, and stand alone.
Is is a subtitle like 'T1 measurement' (line 194)
Line 273: Could you insert a '-' between 2% and 17% ?
p6: figure 5 caption: It was a mistake: 'G and H' can be changed into 'G-I'
Line 653: It was published in 2014 (instead of 2013).