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

Florence Colliez, Marie-Aline Neveu, Julie Magat, Thanh Trang Cao Pham, Bernard Galiez, 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 (EPR) 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.

Clin Cancer Res; 20(21); 5403–11. ©2014 AACR.

Introduction

Tumor hemodynamics has become a key target in preclinical 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 neovascularature 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), $^{13}$F relaxometry (18), or Overhauser enhanced MRI (19), are either invasive or require the injection of a reporter probe, and are currently not clinically...
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 pO2 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). T1 (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 pO2 (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 R1 (1/T1) of water ("oxygen enhanced MRI"; refs. 27–29). T1 demonstrates sensitivity to dissolved oxygen, thereby acting as a potential T1-shortening paramagnetic contrast agent (19). An oxygen-induced increase in the R1 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 ΔR1 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 H2O 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 R1 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 R1, 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%O2/5%CO2) 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 pO2.

To this end, the following questions will be addressed: "Is a change in the R1 (ΔR1) of lipids related to a change in the tumor pO2 (ΔpO2)?"; "Are intrinsic R1 values of lipids related to absolute pO2 values?"; and finally, "Are changes in the R1 of lipids more sensitive than changes in the global R1?"

Materials and Methods

Tumor models

A total of 7 × 10^6 NT2 cells (provided by Elizabeth M. Jaffe, M.D., The Sidney Kimmel Cancer Center at Johns Hopkins, Baltimore, MD) or 10 × 10^6 MDA-MB-231 (LGC Promochem), amplifed in vitro, were collected by trypsinization, washed three times with Hanks Balanced Salt Solution (HBSS), and resuspended in 100 μL 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 ± 1.0°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

$T_1$ measurements.

A segmented IR FISP (Inversion-Recovery Fast Imaging with Steady state Precession) sequence (SSFP FID mode) was used to acquire parametric images of $T_1$ 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 and R1 of lipids) were taken sequentially and used. For that purpose, three MR measurements of each type (global R1) were taken and submitted to carbogen breathing (42). EPR experiments were subsequently performed on the same tumors. We compared 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 hypoxic ($n = 3$) 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 $R_1$ 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 $R_1$ mapping.

A change in the $R_1$ of lipids is related to a change in the tumor $pO_2$

While pooling data from 20 different tumors (10 of each tumor model), we can observe a significant correlation between the $\Delta R_1$ of lipids (MOBILE) and the $\Delta pO_2$ in the same tumors ($P = 0.0059, r = 0.5097$; Fig. 2A), whereas similar analysis using the global $R_1$ does not give a significant correlation (Fig. 2B). Individual changes in the $R_1$ corresponding to an increase in absolute $pO_2$ values, as assessed by EPR oximetry, evidence that (i) the $R_1$ of lipids increased concomitantly (by 2%–17% of relative increase) when the $pO_2$ increased, except for one tumor (2.7% decrease; Fig. 2A) and (ii) the global $R_1$ increased from 0% to 6% with respect to the same $pO_2$ increase, except for two tumors which showed decreases of 0.5% and 7% (Fig. 2B). As regards changes in the $R_1$ with respect to a decline in the $pO_2$, the graphs in Fig. 2 show that (i) the $R_1$ of lipids falls systematically (by 0.1%–13.6%; Fig. 2A) and (ii) the global $R_1$ shows mixed results with both negative (down 4.4%) and positive (up 5.6%) changes in matching tumors (Fig. 2B).
Individual R1 values of lipids are related to absolute pO2 values

To investigate the potentially quantitative properties of MOBILE, mean individual R1 values of lipids were compared with individual pO2 values within each tumor model. For this purpose, all pre- and postchallenge mean values of the R1 of lipids (MOBILE) were plotted together with respect to all corresponding pre- and postchallenge pO2 values (Fig. 3A and B).

Since some tumors underwent both challenges, only one basal value of each parameter (pO2, global R1 and R1 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 R1 values of lipids and absolute pO2 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 R1 and pO2 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 R1 values of lipids and the pO2 values (P = 0.0275, r = 0.3726; Fig. 4). We could not pool the global R1 data from both tumor models because there is no correlation in the NT2 tumor model between pO2 values and global R1 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 R1 of lipids are more sensitive than relative changes in the global R1

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...
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 ($Y = 0.01486 \times + 0.9143$ for Lipids $R_1$ and $Y = 0.01486 \times + 0.9143$ 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 3](image-url) 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.

![Figure 4](image-url) 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.
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 $\Delta R_1$ of lipids is positively correlated with a $\Delta pO_2$ 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,

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–I, 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.
especially for tumor oxygenation lower than 5 mm Hg. It should be noted that tissue type dependency would also be applicable for the global R1. Moreover, on the single MDA-MB-231 tumor model, the global R1 also correlated positively with the absolute pO2 values. Accordingly, MOBILE seems to be more adapted to assessing tumor oxygenation than the parent technique assessing the global R1, in the tumor models included in this study, and is systematically more sensitive than the global R1. Nevertheless, both techniques remain complementary in nature because the origin of the information is different for lipid and global R1, 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), outlining 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 successfully implemented in the clinical setting, it is promising for further clinical monitoring of individual tumor oxygenation to assess the response to antiangiogenic or antivascular 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 have a pending 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

Acknowledgments
The authors thank Philippe Levêque for assistance with statistical analysis.

Grant Support
This study was supported by grants from the Belgian National Fund for Scientific Research (FNRS), the "Fournier-Majoor Foundation", the Joseph Maisin Foundation, the "Actions de Recherches Concertées-Communauté Française de Belgique, Grant No. ARC 09/14-020," and the "Belgian Cancer Plan."

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received December 20, 2013; revised August 7, 2014; accepted August 8, 2014; published OnlineFirst September 10, 2014.

References


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

Florence Colliez, Marie-Aline Neveu, Julie Magat, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-13-3434

Cited articles
This article cites 49 articles, 12 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/20/21/5403.full#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/20/21/5403.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://clincancerres.aacrjournals.org/content/20/21/5403.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.