A MODEL OF REOXYGENATION DYNAMICS OF HEAD-AND-NECK TUMORS BASED ON SERIAL 18F-FLUOROMISONIDAZOLE POSITRON EMISSION TOMOGRAPHY INVESTIGATIONS

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Purpose: To develop a model for reoxygenation dynamic and its relationship to local control after radiotherapy (RT), based on repeated dynamic [18F]-fluoromisonidazole (FMISO) positron emission tomography (PET) examinations in head-and-neck cancer patients.

Methods and Materials: Ten head-and-neck cancer patients were examined with dynamic FMISO PET before RT with 70 Gy and after approximately 20 Gy. Two of these patients had two additional dynamic FMISO scans during treatment. Local recurrence was assessed by computed tomography–based follow-up 8–24 months after RT. Tumor-specific values for the level of FMISO retention $R$ and the vascular perfusion efficiency $P$ were determined with a kinetic compartment model.

Results: Individual $R$–$P$ scattergrams before and during therapy were analyzed, and significant therapy-induced changes in the characteristic $R$–$P$ patterns were observed. A tumor control probability model was derived that involves the tissue parameters $R$ and $P$ and estimates the time to reoxygenation. On the basis of this model, a malignancy value $M$ was introduced and calibrated by a fit to the observed outcome data. Reoxygenation is reflected by the model as a progression to less-malignant tumor types (i.e., smaller values of $M$). In 4 of 6 patients with severe hypoxia, $M$ had decreased after 20 Gy, whereas 2 patients showed increasing $M$. Four patients showed no hypoxia in the pretreatment scan.

Conclusion: A tumor control probability model was developed based on repeated FMISO PET scans during RT. The model combines the local perfusion efficiency and the degree of hypoxia to estimate reoxygenation time. It constitutes a key for hypoxia image-guided dose escalation in RT. © 2007 Elsevier Inc.

Hypoxia, FMISO PET, Tumor control probability, Reoxygenation, Radiotherapy.

INTRODUCTION

Hypoxia assays of individual tumors are increasingly becoming accessible (1–7), and hypoxia-targeted therapies have been proven efficient for increased local control and overall survival (8–10). Given the long-standing tenet of hypoxia-induced radioreistance of the target cells, it comes naturally to direct a dose increment at the volumes that appear positive in hypoxia imaging. The obvious question of how image intensity translates into dose escalation remains (11–14).

Assuming that the hypoxic cell population exhibits a decrease of radiosensitivity by a factor of 2.5–3 (15), it is immediately clear that this cannot be compensated for by dose escalation unless this cell population is rapidly reduced by reoxygenation. Thus, the most relevant quantity for hypoxia image-guided dose escalation (HIDE) is the time from the onset of radiotherapy to reoxygenation, which is equivalent to the number of treatment fractions lost on the radioreistant cells. At first sight, this suggests that a single pretreatment hypoxia image cannot suffice to determine the required extent of dose escalation.

The presence of regions in a tumor where cells can suffer from a lack of oxygen supply is a symptom of an insufficient or deficient vasculature. Hence, despite the complicated processes that occur during normal growth or under therapy (e.g., repopulation, redistribution between cell compartments, and loss of cells either by starvation or as a consequence of therapy), the central question for HIDE is,

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when does the vasculature become sufficient again, either by neovascularization or reduced consumption, or both? Animal experiments show that the phenomenon of hypoxia is an inherent property of cell lines (16) and can far outlast the life span of hypoxic cells (17). The emergence of hypoxia seems to be ingrained in the growth characteristics of a clonogenic population.

If functional imaging reveals the irregularity and coarseness of the tumor vasculature, it may be possible to estimate the time to reoxygenation. In dynamic studies of positron emission tomography (PET) tracer or magnetic resonance/computed tomography (MR/CT) contrast agent uptake, it is possible to obtain a measure of the efficiency of perfusion (determined by the number, caliber, and distribution of blood vessels and the magnitude of blood flow within them) during the influx phase shortly after injection. The degree of hypoxia as given by the uptake of PET tracer is in itself an indication of the coarseness of the vasculature, because hypoxia can only persist in wide pouches between perfused blood vessels. The combination of perfusion efficiency and hypoxia labeling has been shown to carry significant information regarding the success of chemoradiotherapy (18).

In the following report, we develop a model for tumor control that predicts in essence the time to reoxygenation from measurements of perfusion efficiency and hypoxia with the PET tracer $^{[18F]}$-fluoromisonidazole (FMISO). The model was inspired by a series of repeated PET scans of a number of head-and-neck cancer patients undergoing chemoradiotherapy, giving some insight into the changes of both perfusion efficiency and hypoxia. All dynamic PET scans were evaluated with a kinetic compartment model introduced by Thorwarth et al. (19).

**METHODS AND MATERIALS**

**Patients**

The total size of the study was 15 patients. Detailed patient and tumor characteristics have been previously published (18). Each of these patients was examined with fluorodeoxyglucose (FDG) and dynamic FMISO PET before the start of treatment. A subgroup of 10 patients received repeat scans after approximately 2 weeks of treatment (approximately 20 Gy). Patients were treated with radiochemotherapy of 70 Gy applied in 35 fractions. Therapy outcome data were available for all patients. After the end of therapy, patients were reviewed regularly every 3 months with clinical examination, flexible endoscopy, and CT scan when recurrent disease was suspected. Routine CT scans were also acquired 6 weeks and 1 year after therapy was finished.

For a total of 8 patients (Patients 1–4, 7, 9, 10, and 14), one FMISO repeat scan is available, whereas for 2 patients (Patients 6 and 13), three repeat scans could be acquired. Patient 6 had follow-up scans after 20, 50, and 70 Gy, Patient 13 after 10, 20, and 50 Gy. The secondary FMISO PET scans were also acquired using a dynamic acquisition mode. The scans were performed as explained previously (18). The long acquisition times made it difficult to organize the trial and put a large burden on the patients, who were suffering from acute reactions during therapy.

The repeat scans were analyzed with the same kinetic model as the pretreatment scans, and scatter plots as shown below were calculated according to the results of the compartmental analysis (19). The volumes taken into account for the analysis of the repeat scans were defined by the FDG PET–positive areas of the tumors before the start of treatment. Note that tumor size cannot be assessed on an FMISO image.

**Tumor control model**

Naturally, given the population-averaged experimental data, the best a tumor control model including reoxygenation can do is to describe the observations. No attempt at a mechanistic model is made, and all parameters are understood as averages over the patient population.

We start with the common Poisson approximation of tumor control probability (TCP):

$$-\log \text{TCP} = \rho \sum_{i=1}^{n} \exp(-\alpha_{i}D),$$

where $\rho$ is the mean cell density per volume element, $\alpha_{i}$ is the mean cell sensitivity, $D_{i}$ is the dose in volume element $i$, and the sum runs over all volume elements $i = 1, \ldots, n$. The right hand side (rhs) is equal to the expected value of surviving cells in the total tumor volume, henceforth denoted with $\mu_{0}$. If $\mu_{i} = \rho \exp(-\alpha_{i}D_{i})$ is the expected value of surviving cells per volume element, we obtain

$$\mu_{0} = \sum_{i=1}^{n} \mu_{i}.$$ (2)

Assume now that the cells of a given tumor volume element are labeled according to their distance $s$ to the nearest perfused blood vessel. Next, the cells are sorted according to their distance label and collected into bins. Let $\rho h(s)$ be the number of cells in the bin of width $\Delta s$ and mean distance to the next vessel $s$. Clearly, the number of cells will decrease as the mean distance to the next vessel increases: $h(s) \rightarrow 0$ as $s \rightarrow \infty$.

To take into account hypoxia-induced radioresistance, we assume that each bin has a specific mean cell sensitivity $\alpha(s)$ and reoxygenates after a fraction $t_{0}(s)$ of the total number of treatment fractions. Therefore:

$$\mu_{i} = \rho \int_{0}^{s} ds \ h(s) \ \exp(-\alpha(s)t_{0}(s)D_{i} - \alpha_{0}(1 - t_{0}(s))D_{i})$$

$$\approx \rho \exp(-\alpha_{0}D_{i}) \int_{0}^{s} ds \ \exp(-\alpha(s) - \alpha_{0}t_{0}(s)D_{i} + \ln h(s)).$$ (4)

The integral is the excess of cells surviving the treatment because of reduced cell sensitivity. In case no hypoxia is present, the integral is 1. If there is some hypoxia present, the integral becomes rapidly dominated by the bins in which a large number of cells are initially hypoxic and suffer delayed reoxygenation. Assume that these bins are centered around a distance $s_{0}$. In keeping with the well-established saddle-point approximation (i.e., in the limit $D_{i} \rightarrow \infty$), we write

$$\int_{0}^{s} ds \ \exp(-\alpha(s) - \alpha_{0}t_{0}(s)D_{i} + \ln h(s)) \approx \sqrt{\frac{2\pi h'(s_{0})}{|f''(s_{0})|D_{i}}} \exp(-\alpha(s) - \alpha_{0}t_{0}(s)D_{i}),$$ (5)
where \( \alpha_h = \alpha(x_0) \) and \( t_{R_i} = t_{R_f}(x_0) \) are the macroscopically observable quantities of cell sensitivity and reoxygenation time for this volume element. The term \( (2\pi \frac{h^2}{(f''(x_0)D)})^{0.5} \) has a constant value, \( f(s) \) is given by \( f(s) = (\alpha(s) - \alpha_0)t_{R_f}(s) \). In the following, this normalization constant will be neglected, because it can be absorbed in the fit constant \( A \) of the final model (Eq. 7). The rhs is termed the malignancy value \( M_i \) of this volume element. The malignancy value introduced here is purely related to tumor hypoxia and consequential treatment resistance. It does not relate to the potential of a tumor to develop metastases.

The kinetic analysis of FMISO uptake delivers for each volume element a measure of perfusion efficiency \( P \) and of tracer retention \( R \). Previously (18), it was shown that the distributions of \((R, P)\) are quite characteristic for each tumor, with an indication that the presence of high-\( R \) – low-\( P \) volume elements is an unfavorable indicator for treatment response. Here, we assume that (1) the macroscopic cell sensitivity \( \alpha_h \) is proportional to the tracer retention \( R \), and (2) the macroscopic time to reoxygenation \( t_{R_f} \) is proportional to \( 1/(P + P_0) \), suggesting that there exists a maximum time \( 1/P_0 \) after which any volume element will have reoxygenated. The results corroborate this choice.

Finally, we obtain

\[
M_i = \exp(bR_i/(P_i + P_0))
\]

where the dose \( D_i \) to the volume element \( i \) is implicit in the parameter \( b = b^1D/D \). Assuming that \( D_i = D \) everywhere, the final expression for the total number of surviving cells reads

\[
\mu_0 = \rho \exp(-\alpha_0 D) \sum_{i=1}^{n} M_i.
\]

The parameters \( A = \rho \exp(-\alpha_0 D) \), \( b \), and \( P_0 \) were determined by a maximum log-likelihood fit of \( \exp \) \( (- \mu_0) \) to the group of 15 patients, as shown previously (18).

**RESULTS**

The distributions of tracer retention–perfusion efficiency for each patient can be classified as one of three typical scatter patterns. The classic hypoxic tumor shows high tracer retention and lower than average perfusion efficiency values (Fig. 1a). Here, a deficient vasculature creates pockets of severe hypoxia, which may even contain necrotic cells that are not visible on FMISO images. A second class of tumors shows significant tracer retention and more than average perfusion efficiency values (Fig. 1b). These tumors do have a viable vasculature, but oxygen consumption is so high that the supply is not sufficient. These tumors may be more amenable to hypoxia-modifying treatments and may show faster reoxygenation than the first type. Finally, tumors of the third class show no tracer retention, and their perfusion efficiency values are normal to greater than average (Fig. 1c). Previously (18), it was shown that the prognosis for Type 1 is very poor and for Type 2 intermediate, but very good for Type 3.

The observed correlation between the two-dimensional distributions of tracer retention and perfusion efficiency corroborates Assumptions 1 and 2 made in the Methods.
section. Because high levels of tracer retention indicate an unfavorable prognosis, this parameter was assumed to be proportional to the sensitivity parameter \( \alpha_s \) (Assumption 1). Second, the fact that very poor outcome could be associated with patients also presenting with badly perfused tumors indicates an inverse correlation of perfusion efficiency and the time until reoxygenation occurs (Assumption 2).

The fast response of a tumor to radiotherapy could be dominated by two effects. First, because of a deceleration of proliferation the oxygen consumption drops, and as a consequence perfusion-limited hypoxia vanishes. Second, because of an acute inflammatory reaction the blood flow increases, which increases oxygen supply with the same result as above. This turns out to be the reoxygenation pattern of Type 2 tumors. Of the 6 patients classified as Type 2, only 3 had noticeable traces of hypoxia left at 20 Gy, and the perfusion was generally enhanced.

At later times during treatment, the reoxygenation response could be shaped by an overall shrinkage of tumor mass and neovascularization, two effects that can enhance the quality of the vasculature, which is certainly also damaged by radiation. The net effect is somewhat elusive on the basis of current knowledge. For this reason, Type 1 tumors are more interesting study objects. Figure 2 shows the scatter patterns of Patient 6 before treatment, at 20 Gy, and at 50 Gy. The early response leads to increased perfusion efficiency, but the overall reduction of tracer retention is rather small. The 20 Gy scatter pattern resembles a Type 2 tumor. At 50 Gy, hypoxia has almost vanished, resulting in a Type 2–3 scatter pattern.

The example of this patient suggests the hypothesis that the footprint of reoxygenation in the scatter patterns is a progression to less-malignant types. In other words, the malignancy value determined on the basis of dynamic FMISO PET decreases during radiotherapy. The aforementioned and additional unknown mechanisms of reoxygenation and redistribution may in effect increase perfusion efficiency and reduce tracer retention and thus propagate a volume element in the scatter plot from the lower right to the upper left. This propagation would move a volume element from a region of high malignancy to one of lesser malignancy. Hence, the lines of equal malignancy would run orthogonal to the direction of propagation (i.e., from the lower left to the upper right).

This is in fact what the above TCP model tries to capture. From Eq. (6), the lines of constant malignancy have the form

\[
P = \frac{bR}{\log M} - P_0. \tag{8}
\]

The model was chosen such that it produces the simplest possible form of iso-malignancy lines that still describes the
observations. The directions of progression to lower malignancy obtain as

\[ \nabla M = \frac{bM}{P + P_0} \left( \begin{array}{c} -1 \\ R \\ \frac{P}{P + P_0} \end{array} \right) \]  \hspace{1cm} (9)

Figure 3 shows the iso-malignancy lines and the directions of progression for the example patient of Fig. 2.

The parameters of the model were obtained from a maximum likelihood fit to the initial set of 15 patients. The following parameter values were determined from the fit: \( A = 9.92 \cdot 10^{-5}, b = 208.0, \) and \( P_0 = 0.704 \). For each patient, a TCP value was computed. The patients were grouped according to the expected number of surviving cells into four groups. The observed rate of local control is compared with the predicted TCP value of the model in Fig. 4.

The ratio of \( \bar{M} \) before the start of treatment (\( \bar{M}_0 \)) and after 10 fractions (\( \bar{M}_{10} \)) represents the factor by which the expected value of surviving cells is increased because of hypoxia. For the analysis of the mean malignancy \( \bar{M} \), only patients were taken into account that presented \( \bar{M}_0 \geq 1.69 \). This cutoff value corresponds to a reoxygenation time of 2 days, which is assumed to be the magnitude of the associated errors. Of the 10 patients, 4 presented \( \bar{M}_0 \) values below this cutoff (Patients 1, 7, 10, and 13); the mean malignancy values for the remaining 6 patients are shown in Table 1. In four cases (Patients 3, 4, 6, and 14), these results support the hypothesis of a progression to less-malignant tumors during irradiation. The small increase in malignancy observed for Patient 9 is probably due to errors emerging from the data analysis. In contrast, the high \( \bar{M} \)-value determined for the follow-up data of Patient 2 represents an outlier, which may be caused by too sparse dynamic FMISO PET data. For this patient, dynamic data were acquired during a period of only 10 min. This is, in general, too short to accurately image the important regions of the FMISO uptake curves.

### DISCUSSION

The present development sees hypoxia and reoxygenation essentially as a consequence of a deficient vasculature and its response to radiation. Although hypoxia constitutes a severe problem in radiotherapy for a variety of reasons (20), it stems from a deeper cause. The model tries to capture the observable patterns of hypoxia and reoxygenation, both of which are linked to the irregularity of vascularization.

The presented model assumes that the parameter tracer retention is proportional to the cell sensitivity \( h \). A second assumption inversely relates the perfusion efficiency with the time until reoxygenation occurs. The observable patterns of reoxygenation in the repeated patient scans, as shown in Fig. 2, justify these assumptions. Additionally, the facts that the developed TCP model fits the patient outcome data very well and that the values of \( M \) shrink for most patients after the first 2 weeks of therapy support Assumptions 1 and 2 in the Methods section.

The processes occurring as a response to therapy in an individual tumor are many and varied. Histologic studies suggest that any imaginable effect can indeed be found in some specimen (16, 17, 21). Naturally, a population-based TCP model can only describe the net effect of all possible

### Table 1. Mean malignancy values before the start of treatment and after 10 fractions for all patients with pretreatment mean malignancy values \( \leq 1.69 \)

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Pretreatment mean malignancy value</th>
<th>Mean malignancy value after 10 fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>35.3</td>
<td>15,810</td>
</tr>
<tr>
<td>3</td>
<td>17.1</td>
<td>1.16</td>
</tr>
<tr>
<td>4</td>
<td>143.1</td>
<td>71.0</td>
</tr>
<tr>
<td>6</td>
<td>8.39</td>
<td>2.56</td>
</tr>
<tr>
<td>9</td>
<td>1.80</td>
<td>4.56</td>
</tr>
<tr>
<td>14</td>
<td>6.02</td>
<td>1.61</td>
</tr>
</tbody>
</table>
scenarios of hypoxia/reoxygenation on local control. Within the limitations of the study size, this seems to be possible with a single pretreatment hypoxia image.

The reason for this may be that, on average, both the initial degree of hypoxia and the speed of reoxygenation are linked to the irregularity of the vasculature. The patchiness of the intervascular spaces defines the regions where hypoxia can occur. Redistribution of cells between compartments or even a constant loss of the most hypoxic cells do not change anything about the number of cells that are located in a hypoxia-prone region at any given time. Only slower processes like neovascularization or shrinkage of the tumor can change the overall constitution of the vasculature.

In this image, the concepts of acute and chronic hypoxia seem to lose meaning in the context of clinical treatments. Clearly, both are signs of a less than sufficient vasculature, so the likelihood of coincidence is high, especially given the rather large dimensions of PET image voxels. For a model that averages over the total treatment time and a population of patients, all that matters is the mean size of the hypoxic pouches created by the patchy vascularization. The more relevant distinction here is whether this quantity diminishes quickly because of fast reoxygenation, driven by increased supply and reduced consumption of oxygen, or whether reoxygenation occurs by slow processes.

The speed of reoxygenation also impacts the strategy of dose escalation that could be adopted to overcome hypoxia-induced radioresistance and the consequential reduction of cell kill. If dose escalation was applied from the beginning of treatment, a lot of the additional dose administered to the hypoxic region could be wasted on resistant cells if reoxygenation was slow. It could be better to wait with escalation until reoxygenation has occurred and make up for the loss of cell kill toward the end of the treatment, when the dose is more efficient. This strategy runs the risk that reoxygenation may come too late and the dose per fraction become too high.

The model affords predictions about the required dose escalation per volume element. If the strategy of a late boost after reoxygenation is to be adopted, we require for the additional dose $\Delta D$

$$M_i \exp(-\alpha_0 \Delta D) = 1$$

(10)

which leads to

$$\frac{\Delta D + D_0}{D_0} = \log M_i + 1$$

(11)

where $D_0$ is the base treatment dose, in our case 70 Gy, and the cell sensitivity of nonhypoxic tumors $\alpha_0$ equals, say, 0.4. This strategy assumes that the image of initial hypoxia is frozen in the distribution of remaining cells halfway into treatment.

In contrast, a homogeneous boost has to overcome the mean cell sensitivity $\bar{\alpha}$

$$M_i \exp(-\alpha_0 \Delta D) = \exp(-\bar{\alpha} D_0)$$

(12)

so that if we require that

$$\bar{\alpha}(\Delta D + D_0) = \alpha_0 D_0$$

(13)

it obtains

$$\frac{\Delta D + D_0}{D_0} = \frac{\alpha_0 D_0}{\alpha_0 D_0 - \log M_i}.$$  

(14)

This strategy assumes that the reoxygenation is not accelerated by the hypofractionation. Both strategies do not take into account the additional effect of greater fraction sizes, because it is thought that this is of secondary importance given the individual uncertainties of hypoxia images and their interpretation. By means of a Taylor expansion of Eq. 14 for $\log M_i \geq 0$ small, it can be easily seen that both factors agree for small dose escalations. For large dose escalations, the first factor becomes noticeably smaller than the second. The observed range of required dose escalation factors in the present population according to Eq. 14 was between 1 and 1.66.

Some investigators have reported model studies about the efficiency of hypoxia dose escalation when there was a risk of misguiding it due to fluctuations of acute hypoxia or image errors (22). It was found that by far the greatest mishap is missing persistent (i.e., chronic) hypoxia. One may take the liberty to reward this finding: if one misses slowly reoxygenating areas. The present model makes dose escalation predictions on the basis of population-averaged reoxygenation dynamics. Individual patients can deviate from these predictions. As usual, a greater amount of individual information affords a more efficient treatment. Hence, sequential hypoxia images during radiotherapy can be particularly valuable for patients for whom the pretreatment scan predicted slow reoxygenation and consequently high dose escalation factors.

CONCLUSION

A phenomenologic tumor control model including a prediction of reoxygenation speed has been presented and validated with observations of local control in head-and-neck cancer patients after chemoradiotherapy. The essential image information was gleaned from dynamic FMISO PET scans, which provide two individual pieces of information: the perfusion efficiency as seen in the early phase of tracer influx, and late tracer retention. Both quantities relate to the sufficiency and patchiness of the tumor vasculature. The observation of reoxygenation dynamics in this patient population suggests that hypoxia image-guided dose escalation is in fact driven by the degree of irregularity of the vasculature.
REFERENCES