Int. J. Radiation Oncology Biol. Phys., Vol. 79, No. 4, pp. 1188–1195, 2011

0360-3016/$ - see front matter

ELSEVIER

doi:10.1016/j.ijrobp.2010.10.007

**BIOLOGY CONTRIBUTION**

# HYPOFRACTIONATION RESULTS IN REDUCED TUMOR CELL KILL COMPARED TO CONVENTIONAL FRACTIONATION FOR TUMORS WITH REGIONS OF HYPOXIA

David J. Carlson, Ph.D.,*† Paul J. Keall, Ph.D.,* Billy W. Loo, Jr., M.D., Ph.D.,* Zhe J. Chen, Ph.D.,† and J. Martin Brown, Ph.D.*

*Stanford University School of Medicine, Department of Radiation Oncology, Stanford, CA; and
†Yale University School of Medicine, Department of Therapeutic Radiology, New Haven, CT

**Purpose:** Tumor hypoxia has been observed in many human cancers and is associated with treatment failure in radiation therapy. The purpose of this study is to quantify the effect of different radiation fractionation schemes on tumor cell killing, assuming a realistic distribution of tumor oxygenation.
**Methods and Materials:** A probability density function for the partial pressure of oxygen in a tumor cell population is quantified as a function of radial distance from the capillary wall. Corresponding hypoxia reduction factors for cell killing are determined. The surviving fraction of a tumor consisting of maximally resistant cells, cells at intermediate levels of hypoxia, and normoxic cells is calculated as a function of dose per fraction for an equivalent tumor biological effective dose under normoxic conditions.
**Results:** Increasing hypoxia as a function of distance from blood vessels results in a decrease in tumor cell killing for a typical radiotherapy fractionation scheme by a factor of $10^5$ over a distance of 130 $\mu$m. For head-and-neck cancer and prostate cancer, the fraction of tumor clonogens killed over a full treatment course decreases by up to a factor of $\sim 10^3$ as the dose per fraction is increased from 2 to 24 Gy and from 2 to 18 Gy, respectively.
**Conclusions:** Hypofractionation of a radiotherapy regimen can result in a significant decrease in tumor cell killing compared to standard fractionation as a result of tumor hypoxia. There is a potential for large errors when calculating alternate fractionations using formalisms that do not account for tumor hypoxia. © 2011 Elsevier Inc.

**Hypoxia, Hypofractionation, Linear-quadratic model, Cell survival, Radiosensitizer.**

## INTRODUCTION

Tumor hypoxia has been observed in many human cancers and has been shown to correlate with treatment failure in radiation therapy (1). Approximately 90% of all solid tumors have median oxygen concentrations less than the typical values of 40 to 60 mmHg found in normal tissues (2). The decreased oxygenation of tumor cells is a result of structural and functional disturbances of the tumor vasculature that inhibit the normal delivery of oxygen (3). Although hypoxia has been shown to be associated with increased metastasis (4), treatment failure in radiotherapy for tumors with high levels of hypoxia can be attributed primarily to the decreased sensitivity of hypoxic tumor cells to ionizing radiation (5).

The problem of hypoxic radioresistance is reduced through fractionation of the total radiation dose by reoxygenation (6). Although emerging technologies such as stereotactic body radiotherapy (SBRT) provide valuable physical advantages over conventional radiation therapy for patients with solitary tumors (7, 8), hypoxia is expected to be a significant mechanism of radioresistance in SBRT because the total radiation dose is delivered in only a few fractions and the potential for reoxygenation between fractions is reduced.

The purpose of this study is to quantify the effect of radiation fractionation on tumor cell killing, assuming a realistic distribution of tumor oxygenation and full reoxygenation between fractions. Sensitivity of the results to variations in the radiobiologically hypoxic fraction, dose per fraction, and tumor intrinsic radiosensitivity is evaluated. The potential gain in cell killing through administration of a hypoxic cell radiosensitizer is also investigated.

## METHODS AND MATERIALS

### *Development of a cell survival formalism that accounts for a realistic distribution of tumor hypoxia and the temporal pattern of radiation delivery*

The distribution of oxygen in a tumor can be modeled using an arrangement of straight capillaries surrounded by viable tumor cells

Reprint requests to: David J. Carlson, Ph.D., Yale University School of Medicine, Department of Therapeutic Radiology, New Haven, CT 06520-8040. Tel: (203)200-2018; Fax: (203)688-8682; E-mail: david.j.carlson@yale.edu

Supported in part by NIH grant P01 CA067166 (JMB) and American Cancer Society IRG-58-012-52 (DJC).
Conflict of interest: none.
Received March 1, 2010, and in revised form Oct 4, 2010. Accepted for publication Oct 8, 2010.

(9). Oxygen partial pressure $p(r)$ is expressed as a function of radial distance $r$ from the capillary wall (10):

$$p(r) = p_0 \frac{R_{max}^2}{R_0^2}\left(2\ln\frac{R_{max}}{r} - 1 + \frac{r^2}{R_{max}^2}\right), \quad (1)$$

where $p_0$ is the initial oxygen partial pressure adjacent to the capillary wall and $R_{max}$ is the diffusion limit of oxygen in tissue. The parameter $R_0$ is a constant related to the rates of oxygen consumption and diffusion:

$$R_0^2 = R_{max}^2\left(2\ln\frac{R_{max}}{a} - 1\right), \quad (2)$$

where the radius of the capillary $a$ is assumed to be 10 $\mu$m.

As $p(r)$ decreases in a cell, the quantity of lethal radiation-induced damage formed through one- and two-track processes is decreased by a constant and by the square of this constant, respectively. Carlson *et al.* (11) have shown that a single factor can be used to modify intrinsic radiosensitivity in the linear-quadratic (LQ) model. The fraction of cells at radial distance $r$ from the capillary wall that survive a single fraction of radiation dose $d$ may be written as:

$$S(d) = \exp\left(-[\alpha_A/HRF(p)]d - \left[\beta_A G(\lambda,t)/HRF(p)^2\right]d^2\right), \quad (3)$$

where $\alpha_A$ and $\beta_A$ are aerobic radiosensitivity parameters. For low-linear energy transfer (LET) radiation, $\alpha$ represents the quantity of lethally misrepaired and unrepaired double-strand breaks (DSBs), and $\beta$ represents the quantity of lethal exchange-type chromosome aberrations formed through binary misrepair of two separate DSBs (12). The Lea-Catcheside dose protraction factor $G(\lambda,t)$ is dependent on the duration of a single fraction $t$ and the rate of DSB repair $\lambda \equiv \ln(2)/\tau$, where $\tau$ is the DSB repair half-time (13). A hypoxia reduction factor (*HRF*) is introduced to quantify reductions in known radiosensitivity parameters $\alpha_A$ and $\beta_A$ as the oxygen partial pressure in a tumor cell decreases. The *HRF* is defined as the ratio of the dose at a specific level of hypoxia to the dose under fully aerobic conditions to achieve equal cell killing and is predicted by

$$HRF(p) = \frac{mK + p(r)}{K + p(r)}, \quad (4)$$

where $m$ is the maximum *HRF* and $K$ is the oxygen partial pressure at which the *HRF* is half the maximum value.

In a tumor cell population, the fraction of cells at radial distance $r$ per radial distance from the capillary center is

$$f(r) = 2\pi r / \int_a^{R_{hyp}} 2\pi r dr, \quad (5)$$

where $R_{hyp}$ is the diffusion distance at which cells become maximally resistant. The surviving fraction of cells characterized by radial oxygen diffusion $S_{diff}$ can be expressed as

$$S_{diff} = \int_a^{R_{hyp}} f(r)S(r)dr, \quad (6)$$

where $S(r)$ is calculated using Eq. 3. The percentage of cells that survive a single fraction of radiation in a population of maximally resistant tumor cells (*i.e.,* the hypoxic fraction $f_{hyp}$) and tumor cells at intermediate and full oxygen levels $[1 - f_{hyp}]$ is

$$S_{fraction} = f_{hyp}S_{hyp} + (1 - f_{hyp})S_{diff}, \quad (7)$$

where $S_{hyp}$ is the surviving fraction of maximally resistant cells predicted by Eq. 3 using the maximum *HRF*. The total surviving fraction of tumor cells for a treatment of $n$ fractions is

$$S_{total} = (S_{fraction})^n\left(f_{hyp}\exp\left[\gamma_{hyp}(T - T_k)\right] + (1 - f_{hyp})\exp\left[\gamma_{diff}(T - T_k)\right]\right), \quad (8)$$

where $\gamma \equiv \ln(2)/T_d$ is the rate of cellular proliferation, $T_d$ is the cell doubling time, $T$ is total treatment duration, and $T_k$ is the onset or lag-time to cellular proliferation. The formulation above implicitly assumes that full reoxygenation occurs between fractions because the total population of cells returns to the initial oxygen distribution after each fraction. Several clinical studies (14) have observed a general increase in tumor oxygenation during conventional therapy. Such increases work in favor of conventional fractionation and are not relevant to SBRT with a single or a few large doses.

### *Coadministration of a hypoxic cell radiosensitizer*

The radiosensitizing effect of misonidazole has been shown to be dependent on the administered drug concentration and the oxygen partial pressure of the cell population (15). A sensitizer enhancement ratio (*SER*) is introduced that is dependent on drug concentration $c$ and $p(r)$, so that the surviving fraction of cells for a single fraction becomes

$$S(d) = \exp\left(-[\alpha_A \cdot SER(c,p)/HRF(p)]d - \left[\beta_A G(\lambda,t)\cdot SER(c,p)^2/HRF(p)^2\right]d^2\right). \quad (9)$$

The *SER* separates the radiosensitizing effects of misonidazole and oxygen and is defined as the quotient of the drug enhancement ratio and the oxygen enhancement ratio (*OER*) at a known oxygen partial pressure. The *SER* is predicted by

$$SER(p) = \frac{xy + p(r)}{y + p(r)}, \quad (10)$$

where $x$ is the maximum *SER* and $y$ is the oxygen partial pressure at which the *SER* is equal to half of the maximum value.

### *Selection of tumor sites and studies performed*

Clinical data demonstrating a presence of tumor hypoxia in head-and-neck cancer (HNC) (16) and prostate cancer (17) make these tumor sites ideal for this study. The surviving fraction of tumor clonogens is simulated as a function of distance from the blood vessel based on radiosensitivity parameters derived from clinical data and *HRF* values derived from *in vitro* data. The surviving fraction is estimated for an entire radiotherapy course as a function of total number of fractions. Corresponding doses per fraction are calculated to achieve an equivalent tumor biological effective dose (BED) using the standard LQ model without corrections for tumor hypoxia, intrafraction DSB repair, or clonogen repopulation. A conventional HNC treatment of 30 fractions of 2.2 Gy yields a BED of 80.5 Gy for a radiosensitivity of $\alpha_A$ = 0.25 $Gy^{-1}$ and $(\alpha/\beta)_A$ = 10 Gy (18, 19). A conventional prostate cancer treatment of 39 fractions of 2.0 Gy yields a BED of 130 Gy for a radiosensitivity of $\alpha_A$ = 0.15 $Gy^{-1}$ and $(\alpha/\beta)_A$ = 3.0 Gy (20, 21). Assumed time-dependent parameters for HNC ($\tau_{hyp}$ = 1.0 h, $\tau_{diff}$ = 5.0 h, $T_k$ = 21 days, $T_{d_{hyp}}$ = 6 days, $T_{d_{diff}}$ = 3 days) and prostate cancer ($\tau_{hyp}$ = 0.5 h, $\tau_{diff}$ = 2.5 h, $T_k$ = 60 days, $T_{d_{hyp}}$ = 84 days, $T_{d_{diff}}$ = 42 days) were based on the best available data in the literature (20, 22–24). All simulations were performed in FORTRAN 95.