Abstract

Purpose. It has been postulated that the delivery of radiotherapy at ultra-high dose rates (‘FLASH’) reduces normal tissue toxicities by depleting them of oxygen. The fraction of normal tissue and cancer cells surviving radiotherapy depends on dose and oxygen levels in an exponential manner and even a very small fraction of tissue at low oxygen levels can determine radiotherapy response. To quantify the differential impact of FLASH radiotherapy on normal and tumour tissues, the spatial heterogeneity of oxygenation in tissue should thus be accounted for. Methods. The effect of FLASH on radiation-induced normal and tumour tissue cell killing was studied by simulating oxygen diffusion, metabolism, and radiolytic oxygen depletion (ROD) over domains with simulated capillary architectures. To study the impact of heterogeneity, two architectural models were used: (1) randomly distributed capillaries and (2) capillaries forming a regular square lattice array. The resulting oxygen partial pressure distribution histograms were used to simulate normal and tumour tissue cell survival using the linear quadratic model of cell survival, modified to incorporate oxygen-enhancement ratio effects. The ratio (‘dose modifying factors’) of conventional low-dose-rate dose and FLASH dose at iso-cell survival was computed and compared with empirical iso-toxicity dose ratios. Results. Tumour cell survival was found to be increased by FLASH as compared to conventional radiotherapy, with a 0–1 order of magnitude increase for expected levels of tumour hypoxia, depending on the relative magnitudes of ROD and tissue oxygen metabolism. Interestingly, for the random capillary model, the impact of FLASH on well-oxygenated (normal) tissues was found to be much greater, with an estimated increase in cell survival by up to 10 orders of magnitude, even though reductions in mean tissue partial pressure were modest, less than ∼7 mmHg for the parameter values studied. The dose modifying factor for normal tissues was found to lie in the range 1.2–1.7 for a representative value of normal tissue oxygen metabolic rate, consistent with preclinical iso-toxicity results. Conclusions. The presence of very small nearly hypoxic regions in otherwise well-perfused normal tissues with high mean oxygen levels resulted in a greater proportional sparing of normal tissue than tumour cells during FLASH irradiation, possibly explaining empirical normal tissue sparing and iso-tumour control results.

1. Introduction

Within the oxygen depletion hypothesis, it is commonly argued that FLASH radiotherapy reduces normal tissue toxicity by decreasing the level of oxygen in these tissues, thus rendering them more radioresistant due to the OER effect (Dewey and Boag 1959, Spitz et al 2019, Vozenin et al 2019, Pratx and Kapp 2019a, Rothwell et al 2021). Low linear-energy-transfer radiation damages DNA primarily through the generation of free radicals. Oxygen is needed to ‘fix’ this damage, rendering it irreparable; the lower the oxygen levels in tissue, the lower the
impact of radiation (Johansen and Howard-Flanders 1965, Hall and Giaccia 2019). This effect is usually quantified by the OER, defined as the ratio of the dose needed to achieve a given level of cell killing under maximally hypoxic conditions to the dose needed to achieve the same level of cell killing at an oxygen partial pressure \( p \). Viewed as a function of partial pressure, the OER is equal to one at zero tissue partial pressure, \( p = 0 \). As the oxygen partial pressure is increased, OER\( (p) \) initially increases very rapidly, nearly reaching its maximum value (between 2.5 and 3) by 10–15 mmHg (Alper and Howard-Flanders 1956, Wouters and Brown 1997), but changes slowly above this range. The mean oxygen partial pressures \( \bar{p} \) of most normal tissues range from ~20 to 50 mmHg (Ortiz-Prado et al 2019). Based on the slow evolution of OER\( (p) \) with respect to \( p \) at partial pressures above ~10 mmHg, one might thus expect that a substantial (\( \gtrsim 10–40 \text{ mmHg} \)) reduction in partial pressure by radiation would be needed to significantly alter cell killing in normal tissues and hence, reduce normal tissue toxicities. Recently, this has been used to argue that the oxygen depleting effects of FLASH cannot be responsible for its apparent reductions in toxicity, due to comparatively small reductions in oxygen levels (Boscolo et al 2021, Jansen et al 2021).

From both \textit{in vitro} (Weiss et al 1974, Whillans and Raut 1980, Michaels 1986) and \textit{in vivo} (Cao et al 2021) experiments, radiation has been shown to deplete oxygen in a dose-dependent manner by 0.1–0.42 mmHg per Gy of delivered radiation, defining the rate

\[
G \equiv \Delta \bar{p} / D \quad (1)
\]

of radiolytic oxygen depletion (ROD). Here, \( \Delta \bar{p} \) is the change in the mean oxygen partial pressure in the system being studied and \( D \) is the administered dose. ROD occurs whenever the dose delivery time is smaller than the characteristic time scale needed to restore oxygen levels depleted by radiochemical reactions (Weiss et al 1974); \textit{in vitro}, this is the time (~1 s) needed for oxygen to diffuse out of capillaries into surrounding tissue and dose must be delivered in \( \lesssim 1 \text{ s} \) to observe these effects (Pratx and Kapp 2019b). In preclinical \textit{in vivo} experiments, the normal tissue toxicity–reducing effects of FLASH have been observed after single–fraction doses between 10 and 50 Gy (Favaudon et al 2014, Zlobinskaya et al 2014, Levy et al 2020, Montay-Gruel et al 2021). Based on measured \( G \) values quoted above, the lower end of this range is expected to give rise to a reduction in mean tissue oxygen partial pressure by 1–4 mmHg, consistent with measured values by Cao and colleagues, who reported FLASH-induced reductions of ~1 and 2 mmHg in tumours and normal tissues, respectively, after a single 20 Gy dose (Cao et al 2021). These values are much smaller than the magnitude estimated previously (\( \gtrsim 10–40 \text{ mmHg} \)) that would naively be needed to significantly alter the OER in normal tissue. This led Pratx and colleagues to propose that normal tissue hypoxic stem cell ‘niches’ (\( \lesssim 10 \text{ mmHg} \)) are responsible for the radiation response of normal tissues (Pratx and Kapp 2019a, 2019b), since such cells would be expected to be in the range of oxygen levels where changes induced during FLASH would significantly influence cell survival.

This argument hints at an important point: even in nominally well-perfused normal tissues, the vascular network is irregular (Krogh 1919, Bennett et al 1991, Egginton and Gaffney 2010, Baish et al 2011, Kissane et al 2021), and while more efficient at delivering oxygen than the typical tumour vasculature (Nordsmark et al 1994), oxygen distributions still exhibit a substantial degree of heterogeneity (Vaupel et al 1989, Spencer et al 2014, Schneider et al 2019, Kissane et al 2021). Consequently, in well-oxygenated normal tissues (mean partial pressures \( \bar{p} \gtrsim 20 \text{ mmHg} \)), there may be hypoxic regions where the local partial pressure \( p \lesssim 10 \text{ mmHg} \) and hence, FLASH susceptible. \textit{Even if these are proportionately small compared to the normoxic regions, radiation-induced cell survival varies exponentially with the effective dose \( D \cdot \text{OER} \), and the response of tissue to radiotherapy will be determined by these most hypoxic cells.}

The heterogeneity of oxygen partial pressures within tissues is thus a potentially important factor in determining the impact of FLASH. In this work, we simulated its effect by solving the oxygen reaction–diffusion equation (Dasu and Toma-Dasu 2008, Petit et al 2009) including the effect of ROD (Pratx and Kapp 2019a, Rothwell et al 2021) over domains containing capillaries (oxygen sources) distributed randomly and on the vertices of a square lattice, thereby simulating two extremes of geometric irregularity (and hence, oxygen heterogeneity). The resulting oxygenation maps were used to calculate target cell survival in normal and tumour tissues using the linear quadratic model, modified to account for oxygen sensitizing effects. These simulations were run with and without ROD, allowing us to calculate the differential cell survival between FLASH and conventional dose rate radiotherapy (herein referred to simply as ‘conventional radiotherapy’). Our work thus builds on the calculations of Pratx and Kapp (2019a) and Rothwell et al (2021), who carried out related simulations for a single cylindrical capillary (‘Krogh geometry’).

To connect these cell survival results with empirical preclinical studies showing reduced normal tissue toxicities and apparent iso-tumour control (Favaudon et al 2014, Wilson et al 2019, Bourhis et al 2019a) using FLASH, we calculated the ratio of the FLASH and conventional radiotherapy doses needed to achieve iso-cell survival for normal and tumour tissues. These were compared with empirical dose-modifying factors for iso-toxicity, which have been measured in several tissues, including lung, brain (Bourhis et al 2019a, Cao et al 1999, et al 2018, et al 2021). These values are much smaller than the magnitude estimated previously (\( \gtrsim 10–40 \text{ mmHg} \)) that would naively be needed to significantly alter the OER in normal tissue. This led Pratx and colleagues to propose that normal tissue hypoxic stem cell ‘niches’ (\( \lesssim 10 \text{ mmHg} \)) are responsible for the radiation response of normal tissues (Pratx and Kapp 2019a, 2019b), since such cells would be expected to be in the range of oxygen levels where changes induced during FLASH would significantly influence cell survival.

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Table 1. Parameter definitions and values.

| Parameter                                                      | Symbol | Value(s)                  | Reference                                      |
|----------------------------------------------------------------|--------|---------------------------|------------------------------------------------|
| Oxygen diffusivity                                            | \(D_0\) | 2000 \(\mu\)m\(^2\)s\(^{-1}\) | Tannock (1972)                                 |
| Maximum oxygen metabolic rate                                 | \(\epsilon_{\text{max}}\) | 5.15, 40 mmHg s\(^{-1}\) | (Dasu et al. 2003) and supplementary materials |
| Oxygen partial pressure at which metabolism is half its maximum | \(K\)   | 2.5 mmHg                  | Dasu et al. (2003)                             |
| Intrinsic rate of radiolytic oxygen depletion                 | \(G_0\) | 0.2 and 0.4 mmHg s\(^{-1}\) | Weiss et al. (1974), Whillans and Rauth (1980), Michaels (1986). |
| Oxygen partial pressure at which ROD is half its maximum      | \(k_{\text{ROD}}\) | 1 mmHg                    | --                                             |
| Capillary radius                                              | \(r_c\) | 5 \(\mu\)m                | Barratt-Boyes and Wood (1957), Dasu et al. (2003) |
| Capillary oxygen partial pressure (venous end)                | \(p_c\) | 40 mmHg                   | --                                             |
| Intrinsic radiosensitivity                                    | \(\alpha\) | 0.2--0.4 Gy\(^{-1}\)      | Pekkola-Heino et al. (1995), Deschavanne and Fertil (1996), El Shafie et al. (2013) |
| Alpha-beta ratio                                              | \(\alpha/\beta\) | 10 Gy for tumors; 3 Gy for normal tissues | Fowler (2010)                                 |
| Oxygen partial pressure at which the OER is half its maximum  | \(K_m\) | 3.28 mmHg                 | Wouters and Brown (1997)                        |
| maximum OER value                                             | \(\text{OER}_{\text{max}}\) | 3                         | Wouters and Brown (1997)                        |

*Range of values chosen to encompass empirically determined rates of ROD.

Montay-Gruel et al. (2021), and skin (Hendry et al. 1982). The limitations of equating cell survival with toxicity are considered in the Discussion.

2. Methods

2.1. Cell survival analysis

The differential impact of FLASH and conventional radiotherapy was quantified by estimated normal and tumour tissue cell survival fractions (SF’s). SF’s were calculated using the linear–quadratic (LQ) model for a single-fraction of radiotherapy (following most preclinical experimental FLASH regimens), modified to account for OER effects by convolving the LQ expression for survival fraction with the time-dependent distribution \(f(p, t)\) of oxygen partial pressure values \(p\) within the tissue:

\[
SF = \frac{1}{T} \int_0^T dt \int_0^{\infty} dpf(p, t)e^{-\alpha \text{BED}(p, D)},
\]

\[
\text{BED}(p, D) = D \cdot \text{OER}(p) \left(1 + \frac{D \cdot \text{OER}(p)}{(\alpha/\beta) \cdot \text{OER}_{\text{max}}}ight).
\]

Here, is the biologically effective dose corresponding to a dose \(D\), modified by the oxygen partial pressure-dependent OER. Equation (2) generalizes a well-known expression for the oxygen-dependent linear quadratic formula (Wouters and Brown 1997, Petit et al. 2009) to the case where the oxygen distribution \(f(p, t)\) is time-dependent, as happens during FLASH radiotherapy, here taken to be delivered between a time \(t = 0\) and \(T = T\).

The partial pressure dependence of the oxygen enhancement ratio (OER) was approximated as (Howard-Flanders and Alper 1957, Wouters and Brown 1997, Carlson et al. 2006)

\[
\text{OER}(p) = \frac{p \cdot \text{OER}_{\text{max}} + K_m}{p + K_m},
\]

where \(\text{OER}_{\text{max}} = 3\) is the maximum OER value, achieved when \(p \gg K_m\). The partial pressure \(K_m\) at which OER achieves half its maximum value is estimated to lie between 1.9 and 3.28 mmHg (Wouters and Brown 1997, Carlson et al. 2006). \(\alpha\) and \(\beta\) in equations (2) and (3) quantify the linear and quadratic dependencies of radiosensitivity with respect to dose; values for these parameters and all others used in the calculation of SF are shown in table 1.

2.2. Radiobiological parameters

It was assumed that equation (2) applied equally well to normal and tumour tissues, with different values of \(\alpha\) and \(\beta\). For tumours, the ratio \(\alpha/\beta\) was chosen to be 10 Gy, while for normal tissues, it was taken to be 3 Gy, standard values for early- and late-responding tissues (Fowler 2010). Because the values of \(\alpha\) under oxic conditions are highly variable between tumour (Pekkola-Heino et al. 1995, Deschavanne and Fertil 1996, El Shafie et al. 2013) and normal (Deschavanne and Fertil 1996) tissues, we simulated normal and tumour cell

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survival using a range of values: 0.2, 0.3, and 0.4 Gy\(^{-1}\), consistent with empirical in vitro values. Equation (2) also assumed that the density of ‘target’ cells (e.g. tumour clonogens) is uniform with respect to oxygenation.

The heterogeneity of oxygen partial pressures within a tissue was quantified by the distribution \( f(p, t) \), which represents the probability that a given point in space will have an oxygen partial pressure \( p \), a time \( t \) after the start of FLASH. As noted in the Introduction, even though the mean oxygen partial pressure \( \bar{p} = \int_0^\infty dp f(p, 0) \) before FLASH is delivered is sufficiently large in normal tissues that OER(\( \bar{p} \)) \( \sim \) OER\(_{max}\), any irregularities in the spatial distributions of capillaries means that there may be regions at low partial pressure, \( f(p \sim 0, 0) \neq 0 \). These radioresistant regions, for which OER \( \approx 1 \), will ultimately determine SF owing to its exponential dependence on \( D \cdot \text{OER}(p) \) in equation (2). In what follows, we will suppress the time variable \( t \) when discussing the pre-FLASH equilibrium oxygen partial pressure histogram: \( f(p) \equiv f(p, t \leq 0) \).

2.2. Oxygen partial pressure distributions

Oxygen partial pressure distributions \( f(p, t) \)'s were calculated by solving the oxygen reaction-diffusion equation

\[
\frac{\partial p(\vec{r}, t)}{\partial t} = D_{O_2} \nabla^2 p(\vec{r}) - \frac{\epsilon_{\text{max}} \cdot p(\vec{r}, t)}{k + p(\vec{r}, t)} - \frac{(D/T) \cdot G_0 \cdot p(\vec{r}, t)}{k_{\text{ROD}} + p(\vec{r}, t)}
\]

(5)

for the oxygen partial pressure \( p(\vec{r}, t) \) at position \( \vec{r} \) on a two-dimensional domain, subject to

\[
p(\vec{r}, t) = p_c \quad \text{for } \vec{r} \quad \text{on the surface of capillaries (radius } r_c)\text{,}
\]

with \( p_c \) the capillary oxygen partial pressure. Details of the two capillary architecture models are described below.

Equation (5) combines the effects of oxygen diffusion (diffusivity \( D_{O_2} \)) with metabolism (the second term on the right-hand side) and ROD (third term on the right-hand side). Oxygen metabolism is the same as the Michaelis–Menten form used by Dasu and collaborators (Dasu et al. 2003) and Petit and colleagues (Petit et al. 2009), with \( \epsilon_{\text{max}} \) the maximum metabolic rate and \( k = 2.5 \text{ mmHg} \). The ROD term was assumed to similarly obey Michaelis–Menten kinetics; it reduces to the form used by Pratx and collaborators (Pratx and Kapp 2019a) in the limit that \( k_{\text{ROD}} \rightarrow 0 \). In vitro studies have discerned no deviation from a constant ROD as a function of oxygen partial pressure down to several mmHg (Weiss et al. 1974, Whillans and Rauth 1980, Michaels 1986), and yet ROD must vanish as the partial pressure does; correspondingly, we chose a small value for the partial pressure \( k_{\text{ROD}} = 1 \text{ mmHg} \) at which the ROD is half its maximum value. We solved equation (5) with and without the ROD term to simulate the effect of FLASH on oxygen distributions.

Following the approach of Weiss and others (Weiss 1972, Weiss et al. 1974, Pratx and Kapp 2019a), equation (5) assumes that ROD is simply proportional to the dose rate \( D/T \), where \( D \) is the dose delivered in a time \( T \), and \( G_0 \) is the constant of proportionality. More generally, the kinetics of the radiochemical reactions responsible for ROD and oxygen fixation of radiation-induced DNA damage are properly described by second-order reaction equations (Ling 1975, Rothwell et al. 2021). As shown by Ling, however, the results of calculations using these second-order reaction equations approach those using the simpler kinetics in equation (5) for radiation pulses lasting longer than the time scales describing oxygen fixation (\( \tau_{\text{ROD}} \)) and ROD kinetics (\( \tau_{\text{ROD}} \)), both on the order of several microseconds (\( \mu s \)) (Ling 1975, Colliaux et al. 2015). (At the same time, as noted in the Introduction, the pulse duration must be smaller than the time needed for oxygen to diffuse away from capillaries for there to be substantial ROD). For pulsed irradiation, we further require that the time between pulses is much longer than \( \tau_{\text{ROD}} \) (B. Most recent experiments, involving either pulsed or continuous irradiation well-satisfy all these conditions (see table 1 in the review paper by Wilson and colleagues (Wilson et al. 2019)).

It was further assumed that the capillary oxygen partial pressure \( p_c \) was unaffected by FLASH, based on the fact that the hemoglobin oxygen dissociation rate \( \sim 20 \text{ s}^{-1} \) (Chakraborty et al. 2004) is much faster than typical values of \( 1/T \). We thus expect that ROD in blood is immediately compensated for by hemoglobin giving up oxygen to maintain a near-constant partial pressure.

Random two-dimensional capillary domains have been used previously to simulate the often-dysfunctional tumour vasculature (Dasu et al. 2003, Petit et al. 2009). In contrast, normal tissues are often assumed to have a well-organized vasculature, optimized to meet metabolic demands, and modelled as a regular array of non-interacting cylinders (Krogh geometry) (Krogh 1919), including in FLASH studies (Pratx and Kapp 2019a, Rothwell et al. 2021). There is a growing appreciation, however, that the normal tissue vasculature exhibits a substantial degree of irregularity, and that this irregularity may be important for understanding tissue physiology (Egginton and Gaffney 2010, Kissane et al. 2021). The hallmarks of such irregularity are regions of hypoxia and, related to this, a long tail in the distribution \( n(\text{DNC}) \) of the minimum distances between sampled points in the tissue extravascular space and the nearest capillary, i.e. the ‘distance-to-nearest-capillary’ (DNC).
This distribution as well as the closely-related minimum intercapillary distance distribution have been studied extensively in both normal (Rakusan et al 1980, Vetterlein et al 1982, Jobshita et al 1990, Bennett et al 1991, Risser et al 2007, Baish et al 2011, Schneider et al 2019, Kissane et al 2021) and tumour (Risser et al 2007, Baish et al 2011) tissues and both distributions indeed exhibit such a tail at long distances (> 50 μm), indicating the likely presence of small regions of poorly oxygenated regions, even in healthy normal tissues (Schneider et al 2019, Kissane et al 2021). This has been confirmed in measurements of normal tissue oxygen partial pressure distributions, which show the presence of anoxic regions \( f ( p \sim 0) \sim 0 \) even in nominally well-oxygenated tissues such as liver, brain, and spinal muscle (Vaupel et al 1989).

We calculated oxygen partial pressure distributions \( f ( p, t) \) as well as DNC histograms for both randomly distributed capillaries and capillaries distributed on the vertices of a square lattice to represent the two extremes of vascular irregularity used in the literature. Based on the similarity between \( f ( p) \) and DNC histograms calculated using the random capillary model and empirical tumour and normal tissue results, we argue in the Discussion that the random capillary model is a better representation of the vasculature in both these tissue types, although results are presented for both. We emphasize that we are not asserting that the normal tissue vasculature is random, only that it exhibits sufficient irregularity that the predicted oxygen partial pressure distributions and architectural metrics arising from a random capillary model better match with empirical results than e.g. a model representing a regular array of capillaries.

Parameter values used in our simulations are shown in table 1. Of note, simulations were carried out using single-fraction doses of 20 Gy delivered in \( T = 0.4 \) s, two representative values of ROD, \( G_0 = 0.2 \) and 0.4 mmHg Gy \(^{-1}, \) a capillary oxygen partial pressure \( p_c = 40 \) mmHg (Rothwell et al 2021), and maximum oxygen metabolic rates \( c_{\text{max}} \) of 5, 15, and 40 mmHg s \(^{-1} \) for both normal and tumour tissues. Justification for these choices is given below.

Recent in vivo preclinical experiments have investigated the effects of FLASH irradiation using single-fraction doses spanning 10–30 Gy (Zlobinska et al 2014, Montay-Gruel et al 2017, Levy et al 2020, Cao et al 2021); we used 20 Gy as a representative value, also the value used in the experiments of Cao et al (2021), the results of which are most comparable to our own. Assuming \( T = 0.4 \) s gives a dose rate of 50 Gy s \(^{-1} \), which is a typical dose rate at which normal tissue sparing effects have been observed (Wilson et al 2019).

We use the notation \( G_0 \) to distinguish the rate of ROD that enters the oxygen reaction-diffusion equation, equation (5), from the empirically observed values \( G \) defined in equation (1). As noted by Pratx and Kapp, the two values only coincide when oxygen diffusion and metabolism are ignored (Pratx and Kapp 2019a). We use \( G_0 = 0.2 \) and 0.4 mmHg Gy \(^{-1}, \) approximately encompassing the range of measured values in vitro (Weiss et al 1974, Willingham and Rauth 1980, Michaels 1986), where oxygen distributions are fairly uniform spatially and hence, diffusion is less relevant.

The choice of \( p_c = 40 \) mmHg corresponds to the expected venous oxygen tension (Barratt-Boyes and Wood 1957, Ortiz-Prado et al 2019). Although partial pressures drop across capillaries from ~90 to 100 mmHg in arteries to 35–45 mmHg in veins (Barratt-Boyes and Wood 1957, Ortiz-Prado et al 2019), radiation response will depend on the least well-oxygenated components of the tissue, i.e. near the venule ends of capillaries. In the Discussion, we discuss the impact of this assumption on our results in some detail, arguing that alternative choices of \( p_c \) (or any other of the values used in our simulations) do not change the conceptual results of our study. This also includes the maximum rates of oxygen metabolism \( c_{\text{max}} \) assumed here to be 5, 15, and 40 mmHg s \(^{-1} \). The value 15 mmHg s \(^{-1} \) is a classic value for tumours (Thomlinson and Gray 1955, Tannock 1972, Dasu et al 2003, Petit et al 2009), based on measurements of human tumours by Warburg (Thomlinson and Gray 1955). Individual tumours exhibit a range of metabolic ratios, however (Dewhirst et al 1994). Similarly, normal tissues exhibit a range of metabolic activities, with liver and kidney generally exhibiting a higher metabolic rate than the average tumour, brain and intestines exhibiting comparable levels, and resting skeletal muscle exhibiting lower levels (see Vaupel et al 1989) as well as table SM1 in the supplementary materials (available online at stacks.iop.org/PMB/67/115017/mmedia). Further discussion of tumour and normal tissue metabolic levels is given in the supplementary materials section 1, where we argue that 5–40 mmHg s \(^{-1} \) encompasses an appropriate range for most normal tissues.

For a specified value of the areal capillary density \( n_c (=N_c / l^2, \) where \( N_c \) is the number of capillaries and \( l =1 \) mm is the size of the simulation domain), equation (5) was solved using a finite-element technique, having randomly distributed the capillaries over the domain or having placed them on the vertices of a regular square lattice with spacing \( \approx l / \sqrt{N_c} \). Zero-flux Neumann boundary conditions were imposed on the partial pressure \( p(R, t) \) along the edges of the domain boundaries. For each capillary configuration, equation (5) was solved for conventional RT (approximated as \( (D / T) \cdot G_0 = 0; \) from here on, we simply indicate conventional RT as \( G_0 = 0 \)) and for FLASH RT \( (G_0 = 0.2 \) and 0.4 mmHg Gy \(^{-1} \)). The equilibrium (static) conventional RT solution was used as the initial condition \( p(R, 0) \) for the corresponding FLASH calculation, carried out for \( 0 \leq t \leq T \). Figure 1 shows the results of one such calculation for a random capillary array. Following the approach of Petit et al (2009), for the random capillary arrays, the calculation was repeated at least 100 times for different random
capillary placements but fixed \( n_c \), to ensure adequate statistics in the calculation of SF (equation (2)). For calculations involving the lattice capillary array, only a single calculation was needed for each \( n_c \). At discrete timepoints \( t = 0, \Delta t, 2\Delta t, \ldots, T \) separated by the increment \( \Delta t \), the resulting spatial oxygen partial pressure maps \( p(\vec{r}, t) \) were sampled over the 1 mm × 1 mm spatial domains, excluding the outermost regions <100 \( \mu m \) from the boundaries, to generate oxygen partial pressure distributions \( f(p, t) \) for each value of \( n_c \) for conventional and FLASH radiotherapy. This exclusion was done to avoid finite-size effects arising from the zero-flux boundary conditions. For each simulation and each timepoint, 900 × 900 points were thus sampled over the domains, corresponding to one partial pressure data point per \( \mu m^2 \). These datasets were combined to generate a total dataset of partial pressure values for each \( n_c \) and timepoint of size \( N_t = 8.1 \times 10^7 \) (= 900 × 900 × 100). Values of \( n_c \) spanned 8–637 \( \mu m^{-2} \). Examples distributions are shown in figure 2 for three representative values of \( n_c \) for \( t = 0 \) and \( t = T \). The sampled partial pressure datasets were used to calculate SF, approximating the integral as a sum (equation (6)) over the number \( N_t \) of sampled points and a trapezoidal time integration

\[
SF \approx \frac{1}{N_t T} \sum_{j=1}^{N_t} \sum_{i=1}^{N_c} \frac{e^{-\alpha BED p(t_{j-1}, D)} + e^{-\alpha BED p(t_j, D)}}{2} \Delta t, \tag{6}
\]

where \( p(t_i) \) is the \( i \)th element of the sampled histogram dataset at timepoint \( t_i \). \( N_t = T/\Delta t \) is the number of timepoints used in the integration. Because the spatial datasets were large, we used only \( N_t = 5 \) timepoints. At each timepoint \( T \), \( p(\vec{r}, t) \) decays smoothly with time and the numerical error associated with this relatively coarse integration step size is small. Survival fraction curves were computed as functions of the mean tissue partial pressure without ROD (equivalently, pre-FLASH), similarly discretized as shown in equation (7):

\[
\bar{p} = \int_0^\infty dp f(p) \approx \frac{1}{N_t} \sum_{i=1}^{N_t} p_i(0). \tag{7}
\]

Here, \( p_i(0) \) is the \( i \)th element of the sampled histogram dataset without ROD (i.e. \( G_0 = 0 \)). We also calculated the change

\[
\Delta \bar{p} \equiv \frac{1}{N_t} \sum_{i=1}^{N_t} [p_i(T) - p_i(0)] \tag{8}
\]

in mean partial pressure over the course of FLASH; i.e. the difference in partial pressure between the end \((t = T)\) and beginning \((t = 0)\) of FLASH.

2.3. Dose modifying factor

To connect the SF results with empirical normal tissue toxicity and tumour control results, we defined the dose modifying factor (DMF) as

\[
DMF \equiv \frac{20 \text{ Gy}}{D_{\text{conv, iso-SF}}}, \tag{9}
\]

where \( D_{\text{conv, iso-SF}} \) is the single-fraction dose of conventional radiotherapy needed to achieve the same survival fraction ('iso-SF') as 20 Gy of FLASH. Having calculated the SF for 20 Gy and the FLASH oxygen distribution for a given capillary density using equation (6), equation (6) was then solved iteratively using the corresponding
conventional dose-rate oxygen distribution to find the dose $D_{\text{conv, iso-SF}}$ that resulted in the same SF. The DMF was calculated for both tumour ($\alpha/\beta = 10\,\text{Gy}$) and normal tissues ($\alpha/\beta = 3\,\text{Gy}$). Results are shown for the representative metabolic rate $c_{\text{max}} = 15\,\text{mmHg\,s}^{-1}$ and both simulated rates of ROD.

3. Results

3.1. FLASH-induced reduction in oxygen partial pressures

FLASH lowered mean tissue oxygen partial pressures by an amount dependent on the initial mean oxygen partial pressure, the value of $G_0$, and the metabolic rate $c_{\text{max}}$; see figure 3. Tissues with low mean partial pressure $\bar{p}$ values were largely refractory to FLASH, with $\Delta\bar{p}$ approaching zero as $\bar{p}$ did. With increasing partial pressure, $\Delta\bar{p}$ grew, reaching a maximum when $\bar{p} \gtrsim 20\,\text{mmHg}$. For the random capillary array model and intermediate metabolic rate $c_{\text{max}} = 15\,\text{mmHg\,s}^{-1}$, maximum $\Delta\bar{p}$ values were $\sim 3\,\text{mmHg}$ for $G_0 = 0.2\,\text{mmHg\,Gy}^{-1}$ and $\sim 7\,\text{mmHg}$ for $G_0 = 0.4\,\text{mmHg\,Gy}^{-1}$, respectively. For the simulated dose of 20 Gy used in our calculations, these correspond to the maximum ROD rates $G$ slightly below $G_0$. As can be seen in figure 3, $\Delta\bar{p}$ decreased with increasing metabolic rate, since the magnitude of the ROD effect depends on the relative sizes of $(D/T) \cdot G_0$ and $c_{\text{max}}$; see equation (3). Results for square lattice capillary configurations were quantitatively similar, and only results for the random capillary model are shown.

3.2. Impact of FLASH on tumour and normal tissue cell survival

For both models of capillary architecture studied, FLASH led to a pronounced increase in cell survival fraction (SF), depending on the mean tissue oxygen partial pressure $\bar{p}$ values. Example plots of SF’s versus $\bar{p}$ for $\alpha = 0.3\,\text{Gy}^{-1}$ and $\alpha/\beta = 3\,\text{Gy}$ are shown in figure 4 for the three metabolic rates studied ($c_{\text{max}} = 5, 15, 40\,\text{mmHg\,s}^{-1}$), a dose of 20 Gy, and the random (top) and lattice (bottom) capillary architectures. SF curves for the other simulated values of $\alpha$ and $\beta$ were qualitatively similar and are shown in the supplementary materials section 2.

At low $\bar{p}$ values (hypoxic tissue), tissues were again found to be refractory to the impact of FLASH, and the SF curves for conventional and FLASH radiation coincided in the limit $\bar{p} \to 0$. For tumours ($\alpha = 0.3\,\text{Gy}^{-1}$ and $\alpha/\beta = 10\,\text{Gy}$), the difference in SF’s between FLASH and conventional radiotherapy remained $\lesssim 1\,\text{order-of-}

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**Figure 2.** Impact of FLASH on oxygen distributions. Each column shows the partial pressure distributions for three representative capillary densities (indicated at top) during conventional radiotherapy (equivalently, before the beginning of FLASH, at $t = 0$) and FLASH with $G_0 = 0.4\,\text{mmHg\,Gy}^{-1}$ at the end of the FLASH pulse ($t = T$). Simulations were carried out for $c_{\text{max}} = 15\,\text{mmHg\,s}^{-1}$.

The distributions for the random capillary array retain a nonzero fraction of tissue near zero oxygen partial pressure, $f(p < 0.05\,\text{mmHg})$, even for tissues with high mean partial pressure. In contrast, these distributions for square lattice capillary arrays are qualitatively similar and are shown in the supplementary materials section.

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**Figure 3.** Tissues with low mean partial pressures $\bar{p}$ grew, reaching a maximum when $\bar{p} \gtrsim 20\,\text{mmHg}$. For the random capillary array model and intermediate metabolic rate $c_{\text{max}} = 15\,\text{mmHg\,s}^{-1}$, maximum $\Delta\bar{p}$ values were $\sim 3\,\text{mmHg}$ for $G_0 = 0.2\,\text{mmHg\,Gy}^{-1}$ and $\sim 7\,\text{mmHg}$ for $G_0 = 0.4\,\text{mmHg\,Gy}^{-1}$, respectively. For the simulated dose of 20 Gy used in our calculations, these correspond to the maximum ROD rates $G$ slightly below $G_0$. As can be seen in figure 3, $\Delta\bar{p}$ decreased with increasing metabolic rate, since the magnitude of the ROD effect depends on the relative sizes of $(D/T) \cdot G_0$ and $c_{\text{max}}$; see equation (3). Results for square lattice capillary configurations were quantitatively similar, and only results for the random capillary model are shown.

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**Figure 4.** Impact of FLASH on tumour and normal tissue cell survival. For both models of capillary architecture studied, FLASH led to a pronounced increase in cell survival fraction (SF), depending on the mean tissue oxygen partial pressure $\bar{p}$. Example plots of SF’s versus $\bar{p}$ for $\alpha = 0.3\,\text{Gy}^{-1}$ and $\alpha/\beta = 3\,\text{Gy}$ are shown in figure 4 for the three metabolic rates studied ($c_{\text{max}} = 5, 15, 40\,\text{mmHg\,s}^{-1}$), a dose of 20 Gy, and the random (top) and lattice (bottom) capillary architectures. SF curves for the other simulated values of $\alpha$ and $\beta$ were qualitatively similar and are shown in the supplementary materials section 2.
magnitude for $\bar{p} \lesssim 10$ mmHg (the expected range for most tumours), using $c_{\text{max}} = 15$ mmHg s$^{-1}$; see figure SM1 in the supplementary materials.
With increasing $p$, the SF curves separated; for the lattice capillary geometry, a maximum separation was achieved for $p \sim 5$–20 mmHg (depending on parameter values), while for the random capillary model, the curves separated monotonically with increasing $p$. For the random capillary model, the separation was substantial, with normal tissue cell survival increasing by as much as 10 orders of magnitude, depending on the relative magnitudes of $G_0$ and $c_{\text{max}}$ (figure 4).

### 3.3. Dose modifying factors

This differential cell killing manifested itself in tumour and normal tissue dose-modifying factors $\geq 1$, rising from 1 at $p = 0$ to maximum values approaching 1.7 (for $c_{\text{max}} = 15$ mmHg s$^{-1}$ and $G_0 = 0.4$ mmHg Gy$^{-1}$); see figure 5. Similar to the survival fraction results, although the magnitude of the maximum DMF was comparable for the random and lattice capillary models (not shown), the maximum DMF for the lattice model was achieved at relatively low mean partial pressure values, $p \sim 5$–10 mmHg, before decreasing rapidly. In contrast, for the random capillary model, DMF continued to grow with increasing $p$, achieving a maximum for $p \gtrsim 20$ mmHg, the expected range of oxygen tensions for normal tissues. To the extent that tumours have lower $p$ than normal tissues, the impact of FLASH was thus greater for normal tissues than for tumours. Figure 5 shows representative DMF curves for tumour ($\alpha/\beta = 10$ Gy) and normal ($\alpha/\beta = 3$ Gy) tissues using $\alpha = 0.3$ Gy$^{-1}$, $c_{\text{max}} = 15$ mmHg s$^{-1}$, and different values of the rate $G_0$ of ROD. $c_{\text{max}} = 15$ mmHg s$^{-1}$ was assumed here to represent both tumour oxygen metabolism as well as normal tissue at an intermediate metabolic rate; see supplementary materials table SM1.

### 4. Discussion

By depleting tissues of oxygen, FLASH radiotherapy was found to increase cell survival because of the OER effect. The difference in cell survival between FLASH and conventional radiotherapy grew by orders of magnitudes with increasing tissue oxygenation, providing a possible explanation of the observed reductions in normal tissue toxicity and apparent iso-tumour control (Favaudon et al 2014, Wilson et al 2019, Bourhis et al 2019a), since tumours are often found at lower oxygen levels than normal tissues, with mean oxygen tensions typically below 10 mmHg (Hockel et al 1993, Fyles et al 1998, Koong et al 2000, Parker et al 2004, Nordmark et al 2005, Vaupel et al 2007). For completely anoxic tissue (i.e. oxygen partial pressure exactly zero), FLASH cannot further deplete oxygen from tissue and the survival curves for FLASH and conventional radiotherapy coincide.

The substantial impact of FLASH on cell survival in well-oxygenated tissue is the central result of this manuscript, contradicting the naive expectations that oxygen sensitization effects based on mean tissue partial pressures should be small (Praxt and Kapp 2019a), and that oxygen must be depleted completely for the ROD mechanism to contribute significantly to toxicity reductions (Boscolo et al 2021, Jansen et al 2021). The relative increase in radioresistance with increasing oxygenation is a result of the heterogeneity of the oxygen distribution in tissues. A single dose of 20 Gy kills all well-oxygenated cells ($SF \sim 10^{-20}$ for OER = $OER_{\text{max}}$ using e.g. $\alpha = 0.3$ Gy$^{-1}$ and $\alpha/\beta = 3$ Gy) and hence, the survival fraction is essentially the fraction $f (p \sim 0)$ of maximally radioresistant cells at ultra-low oxygen levels. FLASH increases cell survival by increasing the number

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**Figure 5.** Iso-survival fraction dose-modifying factors (DMF’s) for the random (left) and lattice (right) capillary models corresponding to a single-fraction FLASH dose of 20 Gy. Curves are shown for early- and late-responding tissues ($\alpha/\beta = 10$ and 3 Gy, respectively), $\alpha = 0.3$ Gy$^{-1}$, and $c_{\text{max}} = 15$ mmHg s$^{-1}$. The shaded regions in indicates the range of empirical dose-modifying factors, $\sim 1.2–1.8$ (Hendry et al 1982, Bourhis et al 2019a, Montay-Gruel et al 2021), defined in terms of normal tissue iso-toxicity.
of cells in this population. Because \( f \left( p \sim 0 \right) \) is small for well-oxygenated tissues (see e.g. the middle and right panels of figure 2), the increase in \( f \left( p \sim 0, \ t > 0 \right) \) during FLASH is proportionately greater for such tissues and the relative cell survival—FLASH versus conventional radiotherapy—likewise grows with increasing mean partial pressure. The exponential nature of cell survival after radiation means that even a very small fraction of hypoxic cells (e.g. less than 0.5% of the non-FLASH irradiated tissue represented in the right panel of figure 2) is estimated to have partial pressures less than 5 mmHg will have a large impact.

To different extents, this behaviour arose in both the capillary geometry models that we used, the random capillary model as well as the lattice model. However, the effect was far more robust using the random capillary model. For the lattice model, the window of mean tissue partial pressures where FLASH was predicted to result in a substantial sparing effect was substantially reduced compared to the random capillary geometry since the lattice geometry eliminates the possibility of any tissue being overly distant from capillaries. On geometric grounds, for the lattice geometry, \( f \left( p \sim 0, \ t \right) \) will be nonzero only when the maximum distance-to-nearest capillary (DNC) (i.e. the point in the middle of the lattice unit cell) is greater than the critical distance \( R_c \left( \bar{c}_{\max} \right) \equiv \sqrt[4]{D O_{2} R} / c_{\max} \). past a single capillary beyond which the oxygen tension vanishes (Thomlinson and Gray 1955). Equivalently, when the capillary density satisfies

\[
n_c \lesssim \frac{1}{2 R_c^2 (\bar{c}_{\max}^2)} = \frac{c_{\max}}{8 D O_{2} R}.
\]

Here, \( \bar{c}_{\max} = c_{\max} + G_0 (D/T) \) is the approximate effective metabolic rate, accounting for the effects of FLASH (In equation (5), FLASH is formally equivalent to a change in oxygen metabolism in the limit where the Michaelis–Menten parameters are identical, \( k_{ROD} = k \)). For larger capillary densities (equivalently, larger mean tissue partial pressures, \( \bar{p} \)), \( f \left( p \sim 0, \ t \right) \) will be zero for all \( t \leq T \), and the effect of FLASH on cell survival vanishes:

\[
\text{lattice geometry: } \lim_{n_c \to \infty} SF_{FLASH} \to SF_{conv}.
\]

In contrast, for the random capillary model, \( f \left( p \sim 0, \ t \right) \) decays exponentially at large mean tissue partial pressure, but remains nonzero, yielding an exponential separation of the survival curves with increasing tissue oxygenation (\( n_r \)):

\[
\text{random capillary geometry: } \frac{SF_{FLASH}}{SF_{conv}} \sim \exp \left[ \eta \frac{n_r D O_{2} R}{c_{\max}} \left( 1 - \frac{c_{\max}}{\bar{c}_{\max}} \right) \right].
\]

Here, \( \eta \) is a constant of order unity; see the supplementary materials section 3 for a derivation of this result. These respective behaviours—the absence of anoxic regions in the lattice model above a critical capillary density and the convergence of the survival curves for the lattice model and exponential separation for the random capillary model—are apparent in figures 2 and 4.

The question of which model—random capillary or lattice array—better describes the actual normal and tumour vascular architectures is thus an important one, insofar as only the random capillary model predicts a robust increase in the impact of FLASH with increasing tissue oxygenation: as evident from equation (12), the choice of parameter values \( n_r, D O_{2}, R, c_{\max}, \) and \( G_0 \) used in our simulations will affect the rate at which the survival curves separate with increasing oxygenation, but not the existence of such a therapeutic advantage. In contrast, for a regular lattice capillary array, unless equation (10) is satisfied, the advantage will not exist. Note that to satisfy the metabolic demands of the majority of a given normal tissue, one would generally expect that \( n_r \gtrsim 1/2 R_c^2 \) and hence, equation (10) may not be satisfied for normal tissues, with \( f \left( p \sim 0 \right) \) vanishing for physiologically relevant parameter values. As noted above, it is the fact that \( f \left( p \sim 0 \right) \) is nonzero—albeit it, very small—in the random capillary model even at high mean tissue oxygen tensions, \( \bar{p} \gtrsim 20 \), that produces the large separation of survival curves, with FLASH producing a significant tissue sparing effect. Empirical studies have indeed found small but nonzero \( f \left( p \sim 0 \right) \) even in well-perfused normal tissues such as liver and brain where \( \bar{p} \gtrsim 20 \text{ mmHg} \) (Vaupe1 et al 1989), in sharp contrast to the \( f \left( p \right) \)’s calculated using the lattice capillary model (see e.g. the lower panel in figure 2), for which \( f \left( p \sim 0, \ t \right) \) is identically zero for large capillary densities, consistent with equation (10).

This behaviour supports the conclusion that, in terms of the predicted oxygen distributions at least, the random capillary model may be a better model of the normal and tumour tissue vasculature than a regular array model. Further evidence for this can be found by comparing calculated DNC histograms. As noted in the Introduction, vessel morphometry studies have quantified this distribution and the closely-related minimum intercapillary distance distribution in normal (Rakusan et al 1980, Vetterlein et al 1982, Johshita et al 1990, Bennett et al 1991, Risser et al 2007, Baish et al 2011, Schneider et al 2019, Kissane et al 2021) and tumour (Risser et al 2007, Baish et al 2011) tissues. A key hallmark of vascular irregularity is a ‘tail’ in these distributions at long distances (\( \gtrsim 50 \mu m \)), indicating the presence of small regions of poor vascularization. In supplementary materials section 4, we show the results of a calculation of \( n(DNC) \) for a well-perfused simulated normal tissue.
with a random vasculature and compare it with results for tissue with a lattice of capillaries. The former is found to result in an n(DNC) that qualitatively matches empirical normal tissue results, while the latter fails to reproduce the characteristic tail. Hence, we conclude that the random approximation is a likely an adequate model of normal and tumour tissue vasculature to predict the impact of FLASH on both these tissues. We emphasize, however, that differences in vascular architectures amongst normal tissues (e.g. brain versus skin) will result in different normal tissue sensitivities to radiation, and a better understanding of vascular irregularity as well as oxygen heterogeneity in different tissues will help improve our assessment of the magnitude of FLASH’s impact.

The fact that the dose-modifying effects of FLASH are only felt by cells at low local oxygen partial pressures led Pratx and colleagues to hypothesize that normal tissue stem cells in hypoxic ‘niches’ are primarily responsible for the radiation response of normal tissues to radiation (Pratx and Kapp 2019b). Our tissue oxygenation distribution simulations suggest that such hypoxic niches may be present in all tissues, even nominally well-vascularized ones. Whether or not the target cells (normal tissue stem cells in the work of Pratx et al) preferentially populate low-oxygen regions or are uniformly distributed with respect to oxygenation (as assumed in this work), the high OER of cells in these niches will protect them from radiation, characterized by the large enhancement in cell survival fraction.

The magnitude of the impact of FLASH—both on the ROD-induced change $\Delta p$ in mean oxygen partial pressure and the change in cell SF’s—depended on our chosen parameter values. Although a comprehensive assessment of the range of parameter values for which FLASH can have a substantial effect has been undertaken by Rothwell and colleagues, who also used a more detailed model of FLASH kinetics (Rothwell et al 2021), we make two observations here. Using dimensional analysis, the impact of FLASH depends on the magnitudes of the two dimensionless parameters

$$x \equiv n_c D_{0,p} \frac{c_{max}}{\bar{c}_{max}} = \frac{n_c R^2(c_{max})}{4}, \quad y \equiv \frac{(D/T) \cdot G_0}{\bar{c}_{max}}$$

(13)

As noted previously, $x$ is expected generally to be on the order unity for normal tissues, ensuring that most of the tissue is at nonzero oxygen partial pressures (note that this does not preclude the possibility that $f(p = 0) \approx 0$, only that it is a small number, much less than one). For all our choices of parameter values corresponding to expected normal tissue mean partial pressure values, $\bar{p} \gtrsim 20$ mmHg, $x$ varied from ~0.5 to ~3, indicating that our parameter choices were physiologically reasonable. Hence, irrespective of our precise choices of parameter values—$n_c$, $D_{0,p}$, $\bar{p}$, and $c_{max}$—the large separation between survival fraction curves shown in figure 4 is expected to hold for normal tissues, defined here as tissue for which $\bar{p} \gtrsim 20$ mmHg, but more generally as tissue for which $x \gtrsim 1$. This leaves $y$ as the only ‘free’ parameter, unconstrained by physiology. It represents the ratio of the ROD-induced temporal rate of oxygen depletion and the rate of oxygen metabolism. If this quantity is small, then $c_{max}$ can be understood as arising from the persistence of a fraction $f(p \sim 0, \ t \leq T) = 0$ of the tissue that is refractory to FLASH, resulting in the slow increase in $\Delta p$ with respect to $p$ in our simulations, likely mimicking the in vivo situation. In contrast, the relative homogeneity of oxygen in vitro means that there is no such fraction for mean partial pressures above several mmHg’s and the decrease in mean partial pressure is independent of $\bar{p}$.

The fact that $\Delta p$ was predicted to vary with respect to $\bar{p}$ (figure 3) is a consequence of oxygen heterogeneity and is an important differentiating feature between in vitro and in vivo experiments, since oxygen should be much more homogeneously distributed in the former. In several in vitro experiments, $\Delta p$ was found to be nearly independent of the pre-FLASH mean oxygen partial pressure $\bar{p}$ down to several mmHg (Weiss et al 1974, Whillans and Rauth 1980, Michaels 1986, Cao et al 2021). Hence, our choice of values for $c_{max}$ and $G_0$ largely encompassed observed rates of ROD.

Because of the up to 10 order-of-magnitude increase in cell SF’s for well-oxygenated tissues ($\bar{p} \gtrsim 20$ mmHg; figure 4) using the random capillary model, for the range of parameters studied by us, FLASH doses would need to be increased by 10%–70% to achieve the same survival fraction as conventional radiotherapy (figure 5). This is comparable to the range of empirical iso-toxicity dose modifying factors, ~1.2–1.8, observed pre-clinically.
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References
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5. Conclusions

Modelling the heterogeneity in oxygen partial pressures that are expected to arise in tissues, we demonstrated
that FLASH radiotherapy can substantially increase target cell survival as compared to conventional
radiotherapy, even though the reduction in mean tissue oxygen levels was not nearly large enough to completely
deplete tissue of oxygen. This effect was more pronounced for better-oxygenated normal tissues with
proportionately smaller hypoxic fractions than hypoxic tumours, possibly explaining the observed differential
impact of FLASH on normal tissue toxicities and tumour response. Our results highlight the importance of
quantifying intra-tissue oxygen heterogeneity to fully assess the impact of FLASH on tissue radiation response.

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