International Journal of Biological-Medical Science and Technology (IJBiMeS)
Volume 3, Issue 1, Pages 16-25, 2025

# FLASH Radiotherapy: Biochemical Mechanisms, Current Evidence, and Remaining Challenges

**Awat Lotfihagh[1*], Roya Boudaghi Malidarreh[2]**
[1] Department of Physics, University of Texas at Arlington, Arlington, TX 76019, USA
[2]Institute of Physics and Technology, Ural Federal University, Ekaterinburg, Russia

* Corresponding Author : alwf12.kurd@gmail.com

**Article Info:**

**DOI:** 10.22399/ijbimes.20
**Received:** 20 July 2025
**Accepted:** 24 September 2025

**Keywords**
Radiation therapy
Conventional dose-rate
ultra-high dose rates (UHDR)
sparing of normal tissue toxicity

## Abstract

**Background:** FLASH radiotherapy (FLASH-RT) delivers radiation in milliseconds and has consistently reduced normal-tissue toxicity without compromising tumor control. Among proposed biochemical mechanisms, radiolytic oxygen depletion (ROD) remains the most extensively examined, with enhanced radical–radical recombination and tissue antioxidant capacity considered as complementary modifiers.
**Methods:** This review synthesizes findings from experimental, modeling, and computational research to elucidate the physical and biochemical mechanisms underlying FLASH-RT. The analysis highlights how ultrafast dose delivery consumes molecular oxygen faster than it can diffuse back, transiently lowering local $pO_2$ and suppressing oxygen-dependent peroxyl-radical chemistry. It also identifies major limitations in current studies and proposes strategies to address them, including improved $pO_2$ measurement, standardized reporting, and integration of ultrafast imaging technologies.
**Results:** Modeling predicts that oxygen reductions are small at clinically realistic doses but may still have biologically meaningful effects in hypoxic stem-cell niches—microregions within normal tissues responsible for regeneration and particularly sensitive to oxygen fluctuations. The magnitude of ROD depends on baseline oxygenation, total dose, dose-per-pulse, and oxygen-replenishment kinetics. Despite strong theoretical and computational support, progress is limited by incomplete beam and oxygenation reporting and the absence of direct, time-resolved in vivo oxygen measurements at microsecond–millisecond scales.
**Conclusions:** A comprehensive understanding of FLASH-RT requires integrating the roles of oxygen depletion, radical recombination, and antioxidant buffering under physiologic conditions. Determining the relative contributions of ROD, radical recombination, and antioxidant buffering under clinically relevant conditions is essential for rational FLASH-RT optimization. Future research should combine standardized dosimetric and oxygenation reporting with ultrafast, spatially resolved oximetry to probe niche-level oxygen dynamics. Addressing these limitations through targeted methodological advances will be critical for achieving selective normal-tissue protection and guiding the clinical translation of FLASH-RT.

## 1. Introduction

Radiation therapy (RT) is one of the three major pillars of modern cancer treatment, alongside surgery and systemic therapy. It is estimated that over half of all cancer patients will receive RT at some point during their disease course, either as definitive therapy, adjuvant treatment, or palliation of symptoms (Delaney GP, 2005). The therapeutic principle of RT lies in depositing ionizing radiation into tumor tissue to induce irreparable DNA damage, particularly double-strand breaks (DSBs), leading to mitotic catastrophe or apoptosis (Hall EJ, 2018)
Technological advances over the past three decades—including intensity-modulated radiation therapy (IMRT), image-guided RT (IGRT), stereotactic body RT (SBRT), and particle therapy—

have greatly improved the ability to conform high doses to tumors while sparing surrounding organs-at-risk (OARs) (Jaffray DA, 2015; Boudaghi Malidarreh, 2024). Despite these innovations, normal-tissue toxicity remains the principal dose-limiting factor, restricting the curative potential for many patients. Late effects such as fibrosis, vascular damage, and neurocognitive decline can impair long-term quality of life, especially for pediatric and head–neck cancer survivors (R A Rosiello, 1990 Mar; Greene-Schloesser D, 2012; Khabaz, 2018).

The biological effectiveness of RT is not uniform; tumor and normal tissues differ in radiosensitivity, repair capacity, and microenvironmental conditions. In conventional dose-rate (CONV) regimens—delivered over minutes—both tumor and normal cells accumulate DNA damage from sustained radical production in an oxygenated environment, often resulting in comparable levels of sublethal injury per unit dose (Hall EJ, 2018). This lack of selective protection for normal tissues is a key obstacle and has motivated the search for fundamentally different dose-delivery paradigms capable of protecting normal tissue without compromising tumor control.

Over the past decade, FLASH radiotherapy (FLASH-RT) has emerged as a potentially transformative paradigm. FLASH-RT delivers ionizing radiation at ultra-high dose rates (UHDR), with reported mean dose rates typically exceeding ~40 Gy/s for FLASH-RT compared to ~0.01 Gy/s for conventional dose-rate (CONV-RT) irradiation (Favaudon V, 2014; Vozenin MC, 2019; Boodaghi Malidarre, 2020). However, mean dose rate alone does not fully define the FLASH regimen. Complete characterization involves several interdependent physical parameters, including the repetition rate or frequency of pulses, the total number of pulses delivered, the individual pulse width—which may range from microseconds to milliseconds—and the overall irradiation time (Favaudon V, 2014). These factors jointly determine the temporal structure of dose delivery and can influence both the magnitude and reproducibility of the FLASH effect. Preclinical studies in zebrafish, mice, pigs, and cats—spanning electron, proton, and photon modalities—have repeatedly demonstrated a marked sparing of normal-tissue toxicity without compromising tumor control at isodose (Favaudon V, 2014; Vozenin MC, 2019).

The reproducibility of the FLASH effect across species, radiation modalities, and organ systems has driven intensive investigation into its underlying mechanisms. For clarity, we define biochemical mechanisms as those occurring from femtoseconds to milliseconds after radiation energy deposition, and biological mechanisms as those manifesting over cellular- to tissue-level timescales. For the purpose of this review, we focus in particular on the biochemical processes that occur on ultrafast timescales following irradiation, while also considering downstream biological consequences.

From a biochemical perspective, three major processes have been proposed. The first is radiolytic oxygen depletion (ROD), in which the extremely rapid deposition of dose consumes molecular oxygen faster than it can diffuse back into the irradiated region. This transient hypoxia limits the formation of oxygen-dependent peroxyl radicals, reducing oxidative damage in normal tissues. The second is free-radical recombination dynamics, where the high instantaneous density of radicals produced by FLASH increases the likelihood of radical–radical interactions, leading to self-annihilation before they can damage cellular components. The third is redox buffering by antioxidant systems, an early molecular process in which endogenous molecules such as glutathione or catalase neutralize reactive oxygen species before they propagate oxidative injury. This buffering capacity may differ between normal and tumor tissues, potentially contributing to the selective sparing observed in FLASH-RT.

From a biological perspective, several mechanisms have been suggested. Preservation of mitochondrial function under FLASH conditions may reduce ROS production, maintain ATP generation, and limit cell-death pathways such as apoptosis. Maintenance of genomic integrity—through reduced clustered DNA damage and faster resolution of strand breaks—may also protect normal tissues from long-term toxicity. In addition, immune modulation, including the sparing of circulating immune cells and changes in the tumor immune microenvironment, has been proposed as a contributor to both normal-tissue protection and tumor control. This review compiles current evidence mostly for biochemical mechanisms, drawing from in vitro, in vivo, and computational studies, highlighting progress, limitations, and remaining gaps.

## *2. Potential Biochemical Mechanisms*

### *2.1 Radiolytic Oxygen Depletion: From Bulk Tissue to Hypoxic Stem Cell Niches*

Ionizing radiation injures biomolecules by two intertwined pathways. In the direct action, energy is deposited within the target (e.g., DNA bases or the sugar–phosphate backbone), creating radical cations or transient negative ions that fragment bonds and

yield base lesions, single-strand breaks (SSBs), double-strand breaks (DSBs), and occasional DNA–DNA or DNA–protein crosslinks; this component grows with LET(Linear energy transfer), which concentrates ionizations and produces clustered damage that is intrinsically hard to repair (Hall EJ, 2018; Nikjoo H, 1999).

In the indirect action, radiation primarily ionizes water, initiating spur chemistry on the picosecond–microsecond scale and generating short-lived reactive species—especially •OH, $e^-$ aq, and H•—that diffuse a few nanometres to oxidize DNA, proteins, and lipids, and this route accounts for a large fraction of DNA damage (Hall EJ, 2018; Lotfihagh, 2024).

Molecular oxygen amplifies the indirect pathway via the oxygen fixation mechanism: addition of $O_2$ to carbon-centered DNA radicals forms peroxyl/alkoxyl products that "fix" otherwise reversible damage, thereby increasing radiosensitivity (oxygen-enhancement ratio ~2–3 for low-LET) (Gray LH, 1953). Conversely, hypoxia or radical scavengers diminish indirect damage by suppressing peroxyl propagation and converting transient DNA radicals back to native structures (Roots R, 1975). Oxygen is essential for stabilizing transient radicals into cytotoxic peroxyl radicals. Therefore, a temporary reduction in local oxygen concentration can substantially decrease oxidative stress in irradiated tissues. This concept underlies the Radiolytic Oxygen Depletion (ROD) hypothesis.

It has been shown that oxygen present during X-irradiation increased the radiosensitivity of Escherichia coli (E. coli) bacteria, with the magnitude of the effect varying between strains, reinforcing the generality of the oxygen enhancement phenomenon across biological systems (Howard-Flanders, 1956). Oxygen enhancement of radiation damage in plant root meristem cells, showing the effect is not limited to microbial or animal systems. (Favaudon V, 2014) provided landmark in vivo evidence that ultra- high dose-rate (FLASH) irradiation spares normal tissue without compromising tumor control. Using a mouse lung model, they observed significantly reduced fibrosis and inflammation following FLASH compared with conventional dose rate (CONV- RT), despite identical tumoricidal efficacy. Early radiobiology experiments demonstrated that delivering ionizing radiation at extremely high instantaneous dose rates could transiently deplete dissolved oxygen in irradiated media, thereby reducing radiosensitivity (Dewey DL, 1959).

Early experimental findings established that delivering radiation in a single, intense pulse—as opposed to multiple pulses spaced apart—can result in greater cellular survival, an effect attributed to brief, radiation-induced oxygen depletion that temporarily reduces radiosensitivity (Dewey DL, 1959; CD, 1967). Recent in vivo investigations in the context of FLASH radiotherapy reinforce this concept. For example, ultra-high dose-rate whole-brain irradiation in mice markedly reduced hippocampal damage and reactive oxygen species (ROS) production compared with conventional dose rates (Vozenin MC, 2019; Montay-Gruel P, 2017).The proposed mechanism involves a rapid drop in physiological oxygen levels during irradiation, faster than the several seconds to minutes required for replenishment, thereby suppressing peroxyl radical formation and shifting DNA damage pathways toward direct hydroxyl radical attack (Montay-Gruel P, 2017; Wilson JD, 2020). Because DNA repair processes are often more efficient under hypoxic conditions, this transient oxygen depletion is considered a key contributor to the normal tissue sparing observed with FLASH.

In contrast to normal tissue, most solid tumors are already chronically hypoxic due to poor vascularization, chaotic perfusion, and high oxygen consumption rates (Vaupel P, 2016). Because baseline oxygen levels in these regions are already low— ≤2.5 mmHg —there is little scope for further depletion during the brief FLASH irradiation window. This means that the relative radiosensitivity of hypoxic tumor cells should remain essentially unchanged compared with conventional dose rate radiotherapy.

### *2.1.1 Limitations and Opportunities for Further Study*

While unchanged radiosensitivity of hypoxic tumor is supported by radiobiological modeling, direct in vivo measurements of tumor $pO_2$ changes during FLASH are scarce, and the hypothesis has yet to be confirmed conclusively in preclinical or clinical tumor models.

Quantitative modeling indicates that transient oxygen depletion only becomes radiobiologically meaningful when the delivered dose is large enough to measurably lower tissue $pO_2$ and the irradiation is completed at a sufficiently high mean dose rate so that oxygen is consumed faster than it can be replenished. In the simulations by (Petersson K, 2020) separation between normoxic and hypoxic survival curves became apparent only at higher total doses, with higher mean dose rates compressing delivery into timeframes shorter than oxygen recovery. This relationship creates a threshold behavior in which oxygen depletion effects are minimal below certain combinations of dose and dose rate.

Simulations comparing continuous vs pulsed delivery show that if the mean dose rate across the whole exposure is the same, predicted $O_2$ depletion is similar; conversely, very high instantaneous dose rate delivered in widely separated pulses allows $O_2$ to rebound between pulses, erasing the depletion and any ROD-driven sparing. That creates a practical constraint for modulated plans with inter-field or inter-segment pauses on clinical linacs (Petersson K, 2020).

Radiolytic oxygen depletion (ROD) is unlikely to occur under normal atmospheric oxygen levels but becomes more feasible under "physoxic" conditions. Both modeling and experimental data indicate that at ~20% $O_2$ (≈150 mmHg), achieving hypoxic oxygen enhancement ratio (OER) levels would require instantaneous radiation doses in the tens to hundreds of gray—far beyond realistic clinical scenarios. In contrast, typical healthy tissues maintain oxygen levels of 3–7% (≈20–50 mmHg), where relatively small reductions in oxygen can meaningfully influence OER. Supporting this, studies show that breathing carbogen—temporarily increasing brain $pO_2$ —eliminates the neuroprotective benefit of FLASH in mice, suggesting that higher baseline oxygen levels counteract ROD-associated tissue sparing (Montay-Gruel, 2019).

Many xenograft tumors contain a substantial proportion of severely hypoxic or even anoxic cells. Under these conditions, there is little oxygen available to remove, so the difference between FLASH and conventional (CONV) irradiation in terms of tumor radiosensitivity is minimal. In contrast, normal tissues at "physoxic" oxygen levels can still experience oxygen depletion sufficient to alter radiosensitivity, leading to sparing effects. This is consistent with the Petersson model, which predicts that at very low baseline $pO_2$ , oxygen depletion has little impact on the oxygen enhancement ratio (OER)—a finding that matches frequent in vivo observations where normal-tissue sparing occurs without compromising tumor control (Petersson K, 2020).

Modeling of the chemical track structure—the sequence of molecular interactions following electron tracks in irradiated, oxygenated water—shows that although oxygen is consumed during irradiation, the amount removed at clinically realistic doses is far too small to cause the degree of radiosensitization change needed to account for the observed normal-tissue sparing. In other words, the drop in oxygen concentration predicted by these models is insufficient to meaningfully alter the oxygen enhancement ratio (OER) for most normal tissue conditions. This implies that additional rapid chemical processes—such as enhanced recombination of peroxyl radicals—are likely reducing the effective yield of damaging reactive oxygen species (ROS) in FLASH, thereby contributing to the protective effect (Boscolo, 2021).

Normal tissues aren't uniformly oxygenated; many maintain hypoxic stem-cell niches (e.g., intestinal crypt base, hair follicle bulge, bone-marrow endosteum). The "niche" hypothesis suggests that even if clinically used FLASH doses do not measurably deplete oxygen across an entire tissue, they might still cause a brief drop in $pO_2$ within certain already hypoxic pockets. Such a drop could be enough to limit peroxyl-radical fixation and protect the stem cells responsible for driving tissue regeneration. This concept was first proposed by Pratx and colleagues and later developed into micro-scale transport and kinetics models describing radiolytic oxygen use under ultra-high dose rate (UHDR) conditions. These models indicate that the greatest changes in radiosensitivity occur when baseline oxygen is at intermediate levels (physoxia), with minimal effects when oxygen is either very high or extremely low. In theory, this behavior could match the idea of niche-specific protection. In support, many normal-tissue stem-cell zones are known to be relatively hypoxic and play an essential role in repair after injury. However, there is still no

direct experimental evidence showing that UHDR pulses cause a larger, time-resolved oxygen drop in these niches compared to surrounding tissue, and several technical as well as conceptual questions remain unanswered (Guillem Pratx, 2019).

A major obstacle for the idea of niche-specific protection is measurement. At present, there are no tools capable of capturing oxygen changes on the sub-millisecond timescale with spatial resolution fine enough to distinguish individual cells or structures only tens of microns across in a living organism. The most advanced approaches we have—such as EPR oximetry and phosphorescence lifetime probes—have detected only small, dose-dependent oxygen decreases in normal tissues at clinically relevant FLASH doses, typically just a few mmHg. However, these readings are averaged over areas far larger than a stem-cell niche, making it difficult to tell whether a larger, short-lived drop is occurring locally, or whether such a drop would even be required. Reviews examining this "oxygen puzzle" emphasize exactly this technological gap and advise caution in attributing the FLASH effect solely to radiolytic oxygen depletion until ultrafast, micro-scale $pO_2$ measurements can be made directly in vivo (Cao, 2021). Addressing this gap will require the development of ultrafast, high–spatial-resolution oxygen measurement tools capable of capturing transient $pO_2$ changes within tens of microns and on microsecond-to-millisecond timescales during irradiation. Potential approaches include advanced phosphorescence quenching microscopy, two-photon phosphorescence lifetime imaging, ultrafast optical nanoprobes, synchrotron-based microbeam spectroscopy, and next-generation EPR microspectroscopy; these technologies could, in principle, directly test whether FLASH pulses produce niche-specific oxygen depletion in vivo.

A second complication for the niche hypothesis comes from its sensitivity to both dose and beam parameters. Modeling work by Petersson et al. (Petersson K, 2020) and others indicates that oxygen-depletion effects become significant mainly when baseline $pO_2$ is in the intermediate range and when the total dose rate—not simply the instantaneous value—is high enough to exceed the rate of oxygen resupply. At atmospheric oxygen (~150 mmHg), such conditions would require unrealistically large single-fraction doses in the tens to hundreds of gray, whereas at very low $pO_2$ (e.g., hypoxic tumor cores) further depletion produces little change in oxygen-enhancement ratio. This parameter dependence implies that niche-level sparing is unlikely to occur uniformly across tissues, irradiation platforms, or delivery schemes—especially in modulated beam patterns that insert interpulse gaps permitting oxygen recovery.

A third limitation of the niche hypothesis is that the supporting evidence remains indirect. In mouse experiments, increasing tissue oxygenation by having animals breathe carbogen (a high-oxygen gas mixture) reduces or eliminates the neuroprotective benefit of FLASH. This shows that oxygen availability influences the effect, but it does not reveal where in the tissue the decisive oxygen change occurs. In particular, it is unknown whether the change happens inside the small, hypoxic stem-cell niches or more broadly in surrounding, better-oxygenated tissue. Meanwhile, direct measurements of radiolytic oxygen consumption in both living tissue and laboratory models show that, at clinically relevant FLASH doses, the overall oxygen drop across an entire tissue is small—typically just a few mmHg (Scarmelotto, 2022). This means there is currently no direct demonstration of niche-specific oxygen depletion during FLASH. Nonetheless, if oxygen depletion does contribute, its small global magnitude is consistent with the idea that any decisive effect would have to be localized to microregions such as niches.

Also, there is a selectivity concern: if hypoxic normal stem-cell niches gain protection through radiolytic oxygen depletion (ROD), tumor stem-cell niches—which are often hypoxic—might also be inadvertently protected. Several reviews have flagged this theoretical risk and recommend that tumor oxygenation be explicitly considered in early FLASH clinical trials. Modeling studies, such as (Hongyu Zhu, 2022), predict minimal depletion effects in severely hypoxic tumor cores—consistent with the preserved tumor control seen in many xenograft experiments—but caution that better-oxygenated clinical tumors could experience some degree of sparing. This boundary scenario has not yet been excluded experimentally.

Emerging ultrafast oximetry techniques offer promising routes to overcome the current experimental barriers limiting direct observation of

niche-level oxygen dynamics. Advanced optical methods such as two-photon phosphorescence lifetime imaging and phosphorescence-quenching microscopy can achieve microsecond temporal resolution and micrometer-scale spatial precision, enabling real-time mapping of transient $pO_2$ changes within living tissues (Esipova, 2019; Finikova, 2008). Similarly, ultrafast optical nanoprobes and time-resolved electron paramagnetic resonance (EPR) microspectroscopy are being developed to capture rapid oxygen fluctuations during irradiation (Zhou, 2020; Cao, 2021). Integrating these modalities with FLASH-capable irradiation platforms would allow simultaneous dosimetric and oxygen dynamic measurements, directly testing whether transient, niche-specific hypoxia occurs during ultrahigh dose-rate delivery.

Another critical challenge involves the possibility that tumor stem-cell niches—which are often chronically hypoxic—could also benefit from transient oxygen depletion, thereby undermining the selectivity of the FLASH effect. One strategy to mitigate this risk is to combine FLASH-RT with hypoxia-activated prodrugs (HAPs) such as evofosfamide (TH-302) or tirapazamine, which are selectively cytotoxic under low-oxygen conditions (Brown, 2004; Hunter, 2016). Delivering these agents shortly before or concurrently with FLASH could exploit the transient hypoxia induced in tumor tissue to enhance tumor cell kill while preserving the protective effects in normal, better-oxygenated tissues. Additional strategies include pairing FLASH with oxygen-carrying nanoparticles or localized microbubble oxygenation, which could preferentially elevate $pO_2$ in tumors prior to irradiation, reducing the risk of stem-cell protection (Song, 2016; Vaupel P, 2016). Incorporating these combined-modality approaches into preclinical testing will be vital to determine whether FLASH can maintain its normal-tissue sparing advantages without compromising tumor eradication.

### *2. 2 Radical recombination and antioxidant level*

Most radiation damage to biomolecules in low-LET beams is indirect, arising from water radiolysis; roughly two-thirds of the injury is mediated by radiolytically generated ROS rather than by direct ionizations on DNA and proteins. Key primary/secondary ROS produced after radiolysis include •OH, $e_{eq}{}^{-}$, •H, $O2\bullet^{-}$, H2O2. Hydroxyl radical reacts at diffusion-controlled rates with DNA, lipids, and proteins, while O2 converts carbon-centered radicals into peroxyls (ROO•), fixing otherwise reversible damage by preventing radical back-reactions in the presence of oxygen (Ibáñez, 2024).

Early mammalian work already warned that dose-rate effects might not be explained solely by oxygen depletion; proposed that free-radical interactions (recombination/annihilation) could modulate biological outcome independently of bulk oxygen changes

For the same total dose, FLASH and conventional beams create the same number of ionization events overall, but FLASH compresses those events in time, yielding far more radicals per unit time (i.e., higher spur density) than conventional dose rates. In contrast to tumor cells, which typically generate elevated levels of endogenous ROS, normal cells generally possess a stronger antioxidant capacity to counteract these reactive species (Nakamura, 2021). Consequently, normal tissues are likely to accumulate fewer ROO· and ROOH molecules than tumors, which often exhibit weakened antioxidant defenses.

Using a physico-chemical model of water radiolysis, (Labarbe, 2020) formally proposed the free-radical recombination hypothesis, showing that at UHDR the rapid, high-density bursts of radicals increase ROO•↔ROO• (and other) recombination, lowering the *area-under-the-curve* exposure of damaging species and thus sparing normal tissue.

Monte-Carlo and track-structure analyses indicate that overlapping radical tracks at very short pulses and very high instantaneous dose amplify these recombination reactions, mechanistically supporting the model (Baikalov, 2023).

One limitation is that radical recombination alone cannot fully account for tumor cell kill. To bridge the gap between normal-tissue sparing and maintained antitumor efficacy, Hu et al. developed a model incorporating radical–antioxidant–oxygen competition. They proposed that the impact of FLASH differs between tumors and normal tissues because of their distinct antioxidant capacities. Normal tissues, with relatively high antioxidant defenses, can effectively neutralize reactive species,

which, together with radical–radical recombination, enhances protection from radiation damage. In contrast, many tumors possess lower antioxidant reserves or imbalanced redox systems, allowing radicals to persist longer and cause lethal oxidative damage despite partial recombination. Conversely, in certain tumors with unusually high antioxidant pools (e.g., elevated glutathione, GSH), these antioxidants can compete with radicals for reaction partners, reducing radical–radical recombination efficiency and weakening FLASH protection within the tumor (Hu, 2023).

### *2. 2.1 Limitations and Opportunities for Further Study*

Simulation studies by (Baikalov, 2023)indicate that the efficiency with which radiation-induced radicals recombine is highly sensitive to the spatial distribution of chemical spurs along particle tracks. These spurs—nanometer-scale clusters of ionizations and excitations—form the initial sites of reactive species generation following water radiolysis. The degree of spur clustering is dictated by the particle's track structure, which in turn depends on beam quality and linear energy transfer (LET). Low-LET beams, such as high-energy electrons, produce relatively sparse, widely spaced spurs, lowering the probability that radicals will encounter each other and recombine. In contrast, higher-LET beams, such as protons or heavier ions, create densely packed spurs, markedly increasing radical–radical interaction rates and thus recombination efficiency. At FLASH dose rates, where radical densities are already elevated due to ultra-short delivery times, these LET-dependent differences in track structure may cause substantial variation in the magnitude of the normal-tissue–sparing effect observed between electron, proton, and heavy-ion modalities.

Although chemical modeling strongly supports the occurrence of enhanced radical–radical recombination under FLASH conditions, there is currently no direct experimental evidence of this process in normal tissues in vivo. Existing support is derived primarily from indirect biological endpoints—such as observed patterns of normal-tissue sparing—rather than from real-time detection or quantification of radicals themselves.

In fractionated or temporally modulated delivery schemes—such as those produced by scanning beams—the presence of interpulse intervals can be long enough to allow significant ROS decay and partial oxygen replenishment in the irradiated tissue. This temporal spacing can diminish or even eliminate the enhanced radical–radical recombination benefits predicted by single-pulse FLASH models, thereby reducing the magnitude of the normal-tissue–sparing effect (Petersson K, 2020).

### *2. 2.2 Comparative Analysis of FLASH Efficacy Across Beam Types (Electrons vs. Protons) Based on Spur Density Influenced by LET*

The influence of Linear Energy Transfer (LET) on radical recombination and oxygen depletion provides an important physical context for comparing FLASH performance across different radiation modalities. LET determines the spatial distribution of ionizations and thus the density of chemical spurs formed along a particle's track. Low-LET beams, such as high-energy electrons, generate sparse spurs separated by tens of nanometers, leading to limited spur overlap and moderate radical recombination efficiency. In contrast, protons and heavier ions possess higher LET values that produce denser ionization clusters, increasing the likelihood of radical–radical interactions and self-annihilation before diffusion to critical targets (Labarbe, 2020; Baikalov, 2023). Under ultra-high dose rate conditions, this enhanced local radical density may amplify recombination and transient oxygen consumption, potentially intensifying the FLASH sparing effect for protons relative to electrons.

Experimental observations support this theoretical framework. Most preclinical FLASH-RT studies demonstrating robust normal-tissue sparing have employed electron beams (Favaudon V, 2014; Vozenin MC, 2019). More recently, proton FLASH irradiation has shown comparable protection in animal models while maintaining tumor control, suggesting that moderate LET may provide an optimal balance between recombination efficiency and manageable damage complexity (Bourhis J, 2019; Wilson JD, 2020). At still higher LET, such as with carbon ions, the extremely dense energy deposition increases radical recombination but also produces complex DNA lesions and clustered oxidative damage that may counteract any biochemical sparing (Boscolo, 2021). Overall, these findings suggest a non-linear dependence of the

FLASH effect on LET, where low-LET electrons and moderate-LET protons are most likely to achieve favorable biochemical and biological outcomes.

## *3. Conclusion*

FLASH radiotherapy has repeatedly shown the capacity to spare normal tissues without compromising tumor control, yet its fundamental biochemical mechanisms remain a subject of debate. Radiolytic oxygen depletion (ROD) remains a compelling explanation under certain conditions, particularly when baseline $pO_2$ lies within physiological ranges or within hypoxic stem-cell niches responsible for tissue regeneration. In these localized microenvironments, even a brief, small-scale drop in oxygen could limit peroxyl-radical fixation and promote stem-cell survival. Conversely, in tissues that are either highly oxygenated or profoundly hypoxic, current modeling predicts only minor overall depletion at clinically realistic doses. Other processes—such as increased radical–radical recombination and inherent differences in antioxidant capacity—are likely to act concurrently, influencing the net yield of damaging reactive species. Significant gaps persist, including the absence of real-time, high-resolution in vivo oxygen measurements, incomplete characterization of beam parameters, and limited understanding of how dose structure, pulse characteristics, and LET affect these rapid chemical events. Addressing these challenges will require integrating ultrafast, micro-scale oxygen sensing with standardized dosimetric and $pO_2$ reporting. Ultimately, defining the relative contributions of ROD and recombination—particularly in the context of niche-specific protection—will be essential for refining FLASH-RT protocols toward optimal, tumor-selective therapeutic outcomes.

### *3.1 Clinical Protocol Implications*

The mechanistic insights discussed above can inform the design of clinically viable FLASH-RT protocols. Preclinical and modeling data suggest that normal-tissue sparing emerges when the mean dose rate exceeds approximately 40–60 Gy $s^{-1}$, pulse widths remain below about 5 ms, and the total irradiation time is completed within 100 ms (Favaudon V, 2014; Vozenin MC, 2019; Petersson K, 2020). Achieving these conditions ensures that radiolytic oxygen depletion and radical-recombination processes occur faster than tissue oxygen resupply, favoring transient hypoxia in normal but not tumor tissue. Because tissue oxygenation is highly heterogeneous, implementing FLASH clinically will require individualized approaches that combine functional oxygen-mapping modalities—such as BOLD-MRI, EPR, or phosphorescence-lifetime imaging—with adaptive beam delivery to mitigate spatial $pO_2$ variations (Cao, 2021; Scarmelotto, 2022).

Preclinical and modeling evidence further indicate that higher dose-per-pulse values (≥0.1 Gy) and ultrashort pulse widths in the microsecond-to-millisecond range enhance instantaneous radical densities, while minimizing interpulse gaps suppresses oxygen rebound that can counteract ROD-driven sparing (Petersson K, 2020; Labarbe, 2020; Baikalov, 2023). The overall magnitude of these effects depends strongly on baseline oxygenation. Quantitative analyses predict that ROD is most relevant when baseline $pO_2$ is within the physoxic range (approximately 20–50 mmHg), while both very high and very low oxygen levels produce negligible effects (Petersson K, 2020; Boscolo, 2021). Experimental findings corroborate these predictions; for example, increasing tissue oxygenation by carbogen breathing removes the neuroprotective advantage of FLASH in mice, implying that an intermediate oxygen window is required (Montay-Gruel, 2019).

In practice, planning should integrate pre-treatment oxygen mapping and adaptive pulse delivery strategies. Functional oxygen-mapping methods—such as EPR oximetry, BOLD-MRI, or phosphorescence-lifetime imaging—can guide beam modulation by revealing regions of oxygen heterogeneity (Cao, 2021; Scarmelotto, 2022; Vaupel P, 2016). These data can be used to minimize oxygen rebound by employing short, contiguous pulse trains and avoiding long inter-field or inter-segment pauses that allow reoxygenation (Petersson K, 2020; Wilson JD, 2020). For highly heterogeneous tissues, additional interventions such as carbogen breathing, nicotinamide administration, or hyperbaric oxygen may help homogenize oxygen levels, although these approaches must be applied cautiously since elevating baseline oxygen can

reduce ROD-mediated protection (Montay-Gruel P, 2017; Cao, 2021).

Adapting FLASH parameters to tumor type and anatomical site is essential for clinical translation. Organs with moderate $pO_2$ and rich vascular networks—such as the brain or lung—appear most suitable for high-dose-per-pulse regimens that utilize both ROD and radical recombination, consistent with observed neurocognitive protection in animal models (Montay-Gruel P, 2017; Vozenin MC, 2019; Bourhis J, 2019). In contrast, hypoxic or poorly perfused tissues show limited oxygen depletion during FLASH, indicating that protection may arise primarily through radical-recombination mechanisms (Vaupel P, 2016; Petersson K, 2020; Wilson JD, 2020). The degree of radical recombination also depends on linear energy transfer (LET); denser particle tracks in proton or heavy-ion beams may enhance recombination efficiency relative to high-energy electron beams (Baikalov, 2023; Wilson JD, 2020; Bourhis J, 2019).

A practical translational framework can therefore include four steps: (i) pre-treatment oxygen mapping to identify regions that may respond to ROD or recombination; (ii) model-based prediction of achievable oxygen depletion and radical kinetics for machine-specific parameters; (iii) dose-time optimization that meets these mechanistic thresholds while maintaining organ-at-risk constraints; and (iv) prospective reporting of full beam structure and oximetry data in clinical trials (Bourhis J, 2019; Wilson JD, 2020; Cao, 2021; Scarmelotto, 2022). Establishing such site-specific frameworks will be essential for translating FLASH-RT from laboratory discovery to safe and effective clinical implementation.

[1] Baikalov, A.A., 2023. Intertrack interaction at ultra-high dose rates and its role in the FLASH effect, *Front. Phys.*, 11, 1215422. https://doi.org/10.3389/fphy.2023.1215422

[2] Boodaghi Malidarre, R.K., 2020. A feasibility study to reduce the contamination of photoneutrons and photons in organs/tissues during radiotherapy, *Iran. J. Med. Phys.*, 17(6), 366–373.

[3] Boscolo, D.S., 2021. May oxygen depletion explain the FLASH effect? A chemical track structure analysis, *Radiother. Oncol.*, 160, 103112.

[4] Boudaghi Malidarreh, R., 2024. FLASH radiation therapy—Key physical irradiation parameters and beam characteristics, *J. Instrum.*, 19(02), P02035.

[5] Bourhis, J., Montay-Gruel, P., Gonçalves Jorge, P., Bailat, C., Petit, B., Ollivier, J., Vozenin, M.C., 2019. Clinical translation of FLASH radiotherapy: Why and how?, *Radiother. Oncol.*, 139, 11–17.

[6] Brown, J.M., 2004. Exploiting tumour hypoxia in cancer treatment, *Nat. Rev. Cancer*, 4(6), 437–447. https://doi.org/10.1038/nrc1367

[7] Cao, X.Z., Zhang, H., Yang, D., 2021. Quantification of oxygen depletion during FLASH irradiation in vitro and in vivo, *Int. J. Radiat. Oncol. Biol. Phys.*, 110(3), 872–882. https://doi.org/10.1016/j.ijrobp.2020.11.036

[8] Todd, C.D., 1967. Effect of high dose rates on survival of mammalian cells, *Radiat. Res.*, 32, 258–279.

[9] Delaney, G.P., Barton, M.B., Jacob, S., 2005. The role of radiotherapy in cancer treatment: Estimating optimal utilization from a review of evidence-based clinical guidelines, *Cancer*, 104(6), 1129–1137.

[10] Dewey, D.L., Boag, J.W., 1959. Modification of the oxygen effect when bacteria are given large doses of X-rays, *Radiat. Res.*, 11, 587–594.

[11] Goodhead, D.T., 1994. Initial events in the cellular effects of ionizing radiations: Clustered damage in DNA, *Int. J. Radiat. Biol.*, 65, 7–17.

[12] Esipova, T.V., Vinogradov, S.A., 2019. Oxyphor 2P: A high-performance probe for deep-tissue three-dimensional imaging of oxygen concentration, *Nat. Commun.*, 10, 5704. https://doi.org/10.1038/s41467-019-13679-1

[13] Favaudon, V., Caplier, L., Monceau, V., Pouzoulet, F., Sayarath, M., Fouillade, C., 2014. Ultrahigh dose-rate FLASH irradiation increases the differential response between normal and tumor tissue in mice, *Sci. Transl. Med.*, 6(245), 245ra93.https://doi.org/10.1126/scitranslmed.3008973

[14] Finikova, O.S., Lebedev, A.Y., Aprelev, A., Troxler, T., Gao, F., Garnacho, C., 2008. Oxygen microscopy by two-photon-excited phosphorescence, *J. Am. Chem. Soc.*, 130(52), 17178–17179. https://doi.org/10.1021/ja806559k

[15] Gray, L.H., Conger, A.D., Ebert, M., Hornsey, S., Scott, O.C.A., 1953. The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy, *Br. J. Radiol.*, 26, 638–648.

[16] Greene-Schloesser, D., Robbins, M.E., 2012. Radiation-induced cognitive impairment—from bench to bedside, *Neuro Oncol.*, 14(Suppl. 4), iv37–iv44.

[17] Pratx, G., Soto, D., 2019. A computational model of radiolytic oxygen depletion during FLASH irradiation and its effect on the oxygen enhancement ratio, *Phys. Med. Biol.*, 64(18), 185005. https://doi.org/10.1088/1361-6560/ab3761

[18] Hall, E.J., Giaccia, A.J., 2018. *Radiobiology for the Radiologist*, 8th Ed., Wolters Kluwer, Philadelphia.

[19] Zhu, H., Smith, J., 2022. Modeling the impact of tissue oxygen profiles and oxygen depletion

parameter uncertainties on biological response and therapeutic benefit of FLASH, *arXiv*, 2201.10020.

[20] Howard-Flanders, P., Alper, T., 1956. Role of oxygen in modifying the radiosensitivity of E. coli B, *Nature*, 178(4540), 978–979.

[21] Hu, A.Q., Yang, X., Zhang, S., 2023. Radical recombination and antioxidants: A hypothesis on the FLASH effect mechanism, *Int. J. Radiat. Biol.*, 99(4), 620–628. https://doi.org/10.1080/09553002.2023.2174831

[22] Hunter, F.W., Wang, J., 2016. Hypoxia-activated prodrugs: Paths forward in the era of personalised medicine, *Clin. Cancer Res.*, 22(15), 3432–3438. https://doi.org/10.1158/1078-0432.CCR-15-3108

[23] Ibáñez, B.M., Martinez, E., 2024. Molecular insights into radiation effects and protective mechanisms: A focus on cellular damage and radioprotectors, *Curr. Issues Mol. Biol.*, 46(11), 12718–12732.

[24] Jaffray, D.A., Gospodarowicz, M.K., 2015. Radiation therapy for cancer, *Cancer: Disease Control Priorities*, 3, 125–140.

[25] Khabaz, R.B., 2018. Estimation of photoneutron dosimetric characteristics in tissues/organs using an improved simple model of linac head, *Appl. Radiat. Isot.*, 133, 88–94.

[26] Labarbe, R.H., Hotoiu, L., Testard, I., 2020. A physicochemical model of reaction kinetics supports peroxyl radical recombination as the main determinant of the FLASH effect, *Radiother. Oncol.*, 153, 303–310. https://doi.org/10.1016/j.radonc.2020.02.018

[27] Montay-Gruel, P., Petersson, K., 2017. Irradiation in a flash: Unique sparing of memory in mice after whole brain irradiation with ultrahigh dose rate, *Radiother. Oncol.*, 124(3), 365–369.

[28] Montay-Gruel, P., Acharya, M.M., 2019. Long-term neurocognitive benefits of FLASH radiotherapy driven by reduced reactive oxygen species, *Proc. Natl. Acad. Sci. U.S.A.*, 116(22), 10943–10951.

[29] Nakamura, H., Arai, T., 2021. Reactive oxygen species in cancer: Current findings and future directions, *Cancer Sci.*, 112(10), 3945–3952.

[30] Nikjoo, H., O'Neill, P., 1999. Quantitative modeling of DNA damage using Monte Carlo track structure, *Radiat. Environ. Biophys.*, 38, 31–38.

[31] Lotfihagh, A., 2024. Investigating the DNA damage patterns under high linear energy transfer (LET) radiotherapy using reactive molecular dynamics (RMD) simulation (AAPM 2024), Los Angeles Convention Center, Los Angeles, CA, USA. https://aapm.confex.com/aapm/2024am/meetingapp.cgi/Paper/12962

[32] Petersson, K., Adrian, G., Butterworth, K., McMahon, S., 2020. A quantitative analysis of the role of oxygen tension in FLASH radiation therapy, *Int. J. Radiat. Oncol. Biol. Phys.*, 107, 539–547.

[33] Rosiello, R.A., Witschi, H., 1990. Radiation-induced lung injury, *Clin. Chest Med.*, 11(1), 65–71.

[34] Roots, R., Okada, S., 1975. Estimation of lifetimes and diffusion distances of radicals involved in X-ray-induced DNA strand breaks or killing of mammalian cells, *Radiat. Res.*, 64(2), 306–320.killing of mammalian cells. . *Radiat Res*, 64(2):306-320.

[35] Scarmelotto, A., Della-Corte, A., 2022. The oxygen puzzle in FLASH radiotherapy: A comprehensive review and experimental outlook, *Cancer Lett.*, 546, 215857.

[36] Song, G., Liang, C., 2016. Perfluorocarbon-loaded hollow $Bi_2$ $Se_3$ nanoparticles for timely oxygen supply and photoacoustic imaging–guided enhanced radiotherapy, *Adv. Sci.*, 3(8), 1600364.

[37] Vaupel, P., Mayer, A., 2016. Hypoxia in cancer: Significance and impact on clinical outcome, *Cancer Metastasis Rev.*, 35, 225–239.

[38] Vozenin, M.C., de Fornel, P., Petersson, K., 2019. The advantage of FLASH radiotherapy confirmed in mini-pig and cat-cancer patients, *Clin. Cancer Res.*, 25(1), 35–42.

[39] Wilson, J.D., Hammond, E.M., Higgins, G.S., Petersson, K., 2020. Ultra-high dose rate (FLASH) radiotherapy: Silver bullet or fool's gold?, *Front. Oncol.*, 9, 1563. https://doi.org/10.3389/fonc.2019.01563

[40] Zhou, L., Zhang, Z., 2020. Time-resolved EPR spectroscopy for probing transient oxygen microenvironments under pulsed irradiation, *J. Phys. Chem. B.*, 124(34), 7451–7460. https://doi.org/10.1021/acs.jpcb.0c04852