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A gap analysis integrating In vitro - Research in HDR interventional radiotherapy (Modern Brachytherapy): Challenges, limitations, and future directions

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1. Introduction

Interventional radiotherapy (modern brachytherapy - BT, IRT) is a well-established modality for delivering localized radiation therapy, widely used in the treatment of various cancers, including gynecological, skin, prostate, and head and neck malignancies (Fionda et al., 2024) By placing radioactive sources in close proximity to or within the tumor, IRT achieves a highly conformal dose distribution, minimizing exposure to surrounding healthy tissues (Chargari et al., 2019). IRT can be performed using either low-dose rate (LDR) or high-dose rate (HDR) sources; however, most preclinical studies published until the last decade have predominantly focused on LDR techniques. Nowadays, clinical practice and technological evolution have led to a near-universal shift toward HDR sources, which enable greater control overdose delivery, improved treatment efficiency, and refined dosimetric planning. While the clinical efficacy of IRT is well-documented, preclinical studies remain essential for optimizing HDR-specific treatment protocols, elucidating radiobiological mechanisms, and fostering innovation in therapeutic strategies aligned with contemporary clinical practice.

In the broader landscape of cancer treatment, IRT plays a key role, particularly in cervical, skin, and prostate cancer, as well as in post-operative radiotherapy or reirradiation for locally recurrent tumors. Its defining characteristic lies in the combination of a high radiation dose, a limited target volume, and a short treatment duration, unlike external beam radiotherapy (EBRT). Given the high dose per fraction and steep dose gradients, incorporating radiobiological principles is essential to optimize treatment planning and enhance biological effectiveness (Stewart et al., 2025). Additionally, it is acknowledged that there are radiobiological differences between brachytherapy and external beam radiotherapy that must be taken into account (Annede et al., 2020).

In vitro models play a fundamental role in radiotherapy research, especially in advancing the understanding of IRT-specific radiobiology (RB). By providing controlled experimental conditions, they enable the investigation of cellular responses to radiation exposure, offering insights into mechanisms such as DNA damage, cell cycle regulation, cell death, radiation-induced stress responses and local immunomodulation. Furthermore, in vitro studies enable the evaluation of radiosensitizers, radioprotectors, and combination therapies, paving the way for improved treatment efficacy and reduced toxicity (Annede et al., 2020).

Recent technological innovations, including the development of microfluidic systems and 3D-printed irradiation platforms, have

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expanded experimental capabilities, while the increasing interest in three-dimensional (3D) culture models such as spheroids and organoids reflects the growing demand for physiologically relevant models in radiobiological research (Chermat et al., 2024). However, despite these advances, significant gaps persist in the current literature. Existing in vitro studies in HDR-IRT remain predominantly limited to two-dimensional monolayer cultures, which fail to replicate critical features of the tumor microenvironment, including hypoxia, extracellular matrix interactions, cellular heterogeneity, and immunomodulation. Furthermore, biological endpoints are often confined to basic cytotoxicity assays, with limited mechanistic insight into sublethal DNA damage, oxidative stress, or cell cycle dynamics. Studies investigating the immune-related consequences of radiation exposure, as well as the bystander effect, an established non-targeted phenomenon in RB, are notably scarce. Additionally, while dosimetric modeling is frequently performed using Monte Carlo simulations, it is rarely integrated with advanced biological systems.

A substantial body of literature exists on in vitro applications of IRT with LDR sources, with most studies relying on traditional 2D cell culture models (Brenner, 1997; Tubin et al., 2023). Over the years, however, there has been a growing shift toward the use of 3D culture systems, such as spheroids and organoids, as well as HDR sources, which now represent the clinical standard in IRT. While radiobiological data derived from LDR-based studies have laid important groundwork, these findings cannot be directly extrapolated to HDR applications due to fundamental differences in dose rate, fractionation, and cellular response dynamics (King, 2002; THE GEC ESTRO HANDBOOK OF). This highlights the need for dedicated in vitro studies specifically designed for HDR-IRT settings, in order to ensure that biological effects are accurately characterized and translated into clinical practice. The 2D and 3D in vitro models differ substantially in their ability to replicate the biological complexity of *in vivo* tumors. While 2D monolayers are easy to manipulate and widely used, they lack critical features such as tissue architecture, gradients of oxygen and nutrients, and cell–matrix interactions. In contrast, 3D models more closely mimic the tumor microenvironment, capturing spatial organization, hypoxia, and cellular heterogeneity, factors that can significantly influence radiation response and therapeutic efficacy (Raitanen et al., 2023).

While several reviews have addressed radiobiology in IRT more broadly, our analysis is the first to systematically focus on in vitro studies using HDR sources (Chargari et al., 2019; Stewart et al., 2025). Findings from LDR-derived studies, which dominate existing literature, cannot be directly extrapolated to HDR regimes because of fundamental differences in dose rates, dwell-time microdosimetry, and photon energy spectra (Richards et al., 2022). Moreover, recent engineering advances, such as 3D microfluidic "brachytherapy-on-a-chip" platforms, provide promising tools for experimental modeling but do not yet address the methodological inconsistencies and limited cellular evidence specific to HDR (Chermat et al., 2024). By systematically mapping available HDR in vitro studies, this gap analysis highlights the scarcity of robust experimental data and emphasizes the urgent need for standardized methodological frameworks tailored to HDR-specific conditions.

This review examines the current landscape of in vitro studies utilizing HDR-IRT sources, analyzing the scope of research across different studies by distinguishing their primary endpoints and the radiation sources employed. By critically evaluating the methodologies used and their translational relevance, we provide insights into how in vitro models contribute to the advancement of IRT applications in oncology. Furthermore, we highlight the notable lack of comprehensive information and systematic characterization of in vitro studies in the current literature, which limits a thorough understanding of their role in supporting clinical innovation.

2. Materials and methods

A comprehensive literature search was conducted in PubMed and Scopus to identify in vitro studies involving high-dose-rate interventional radiotherapy (HDR-IRT). The search strategy incorporated a combination of keywords, synonyms, and acronyms related to HDR brachytherapy, including: ("HDR" OR "high dose rate") AND *("brachytherapy" OR "IRT" OR "BT" OR "interventional radiotherapy") AND ("*in vitro*" OR "cell culture" OR "cell line") as shown in* Fig. S1.

No restrictions were applied on publication date until March 2025. PubMed and Scopus were selected because together they provide comprehensive coverage of clinical and preclinical studies in radiotherapy, ensuring adequate representation of HDR brachytherapy and radiobiology literature.

To enable a quantitative comparison with low-dose-rate (LDR) approaches, an additional search was performed in the same databases using the query: ("LDR" OR "low dose rate") AND ("brachytherapy" OR "BT" OR "Interventional Radiotherapy") AND ("in vitro" OR "cell culture" OR "cell line").

A total of 14 articles were identified through the literature search. However, after reviewing the abstracts, 4 articles were excluded as they did not directly investigate cellular models: while these studies addressed radiobiological aspects, their primary focus was not on in vitro IRT applications. In addition, non-English language papers were excluded from the analysis.

The remaining 10 studies were analyzed based on the type of HDR-IRT source used, the cell lines employed, where defined, the primary research methodology of the study, and the endpoints. Additionally, studies were examined to determine whether they reported quantitative biological responses, dosimetric parameters, or functional biochemical analyses.

Regarding endpoint classification, cytotoxicity includes all studies assessing cell viability through cytotoxic assays. Radiosensitization refers to studies investigating the effectiveness of radiosensitizing agents in enhancing the cellular response to radiation. The α/β ratio category groups studies that focus on evaluating the radiosensitivity of specific cell lines or developing models to estimate α/β ratios under different experimental conditions. In addition, all articles involving dose distribution simulations and experimental setup validations have been classified under the Monte Carlo Dosimetry category, as they primarily focus on the physical modeling of irradiation conditions rather than on biological outcomes: ROS levels, bystander effects, and cell cycle dynamics.

3. Results

Table 1 offers a structured synthesis of the selected studies, outlining their HDR source, research methodology, investigated endpoints, and employed cell lines, while Fig. 1 provides an overview of the distribution of study methodology within the field.

A total of 10 studies published were identified as relevant to in vitro IRT using HDR sources.

The search using the keywords "LDR" AND "brachytherapy" AND "in vitro" identified a total of 78 articles.

Most employed Iridium-192 (^{192}Ir, Ir-192) as the radiation source, with one study incorporating in addition Cobalt-60 (^{60}Co) and Cesium-137 (^{137}Cs), reflecting the continued clinical reliance on ^{192}Ir in experimental settings.

The predominant research methodology among these studies was radiobiological investigation, comprising seven of the ten, while the remaining three emphasized Monte Carlo-based dosimetry modeling.

Within the RB-focused studies, multiple endpoints were explored, most commonly cytotoxicity, which was assessed in four works through viability or clonogenic assays to evaluate radiation effects across different cell lines.

Concerning cytotoxicity, four studies provide a detailed characterization of the cellular responses to HDR IRT across different tumor and normal cell lines, under a variety of experimental conditions. Talik et al. evaluated the combined effects of HDR irradiation with bismuth oxide nanoparticles (BiONPs) and cisplatin in breast cancer cell lines (MCF-7

Table 1
Summary of in vitro HDR-IRT studies, with publication year, radiation source, research metodology, study endpoints, and cell line.

Year	Authors (PMID)	HDR Source	Publication Topic	Research Metodology	Endpoint	Key Findings	Cell Lines
2025	Araujo et al. (Araujo and Fonseca, 2025) (40024052)	192Ir	Monte Carlo radiosensitization study	Dosimetry	Dosimetry, Radiosensibilization	MC model predicts dose enhancement for AuNPs.	Not defined
2025	Thayer-Freeman et al. (Thayer-Freeman et al., 2025) (40062322)	60Co 137Cs 192Ir	Radiobiology dose modeling	Meta-Analysis	α/β Ratio Meta-Analysis	Wide α/β variability affects EQD2.	Cervical Cancer cell lines (14 squamous cell carcinomas and 9 adenocarcinomas).
2024	Silvus et al. (Silvus et al., 2024) (38964977)	192Ir	Monte Carlo study	Dosimetry	Dosimetry	2D platform improves HDR dose accuracy.	Not defined
2022	Chow et al. (Chow et al., 2022) (35086068)	192Ir	Radiobiology	RB in vitro	α/β Ratio	Lower α/β alters EQD2 estimates.	CaSki, C-33A, SiHa, and SW756
2021	Geraldo et al. (Geraldo et al., 2021) (33773203)	192Ir	Monte Carlo study	Dosimetry	Dosimetry	HDR dose deviations	Not defined
2021	Li et al. (Li et al., 2021) (33590493)	Not Available	Radiobiology	RB in vitro	α/β Ratio	UMA model improves EQD2 prediction	Multiple cancer cell lines
2020	Zainudin et al. (Bystander Effect Induced in Breast, 2020) (32637376)	192Ir	Radiobiology	RB in vitro	Cytotoxicity, Bystander Effect and ROS levels	HDR triggers bystander effect and ROS	MCF-7 breast cancer cells and hFOB 1.19 9 normal osteoblast cells
2019	Talik Sisin et al. (Talik et al., 2019) (31908451)	192Ir	Radiosensitizer and radiobiologic study	RB in vitro	Radiosensibilization, Cytotoxicity and ROS levels on	BiONPs and cisplatin enhance HDR radiosensitization	breast cancer cell lines (MCF-7, MDA-MB-231) and normal fibroblast cell line (NIH/3T3)
2018	Shahhoseini et al. (Shahhoseini et al., 2018) (30310276)	192Ir	Radiosensitizer and radiobiologic study	RB in vitro	Radiosensibilization and Cytotoxicity	eBx shows stronger dose enhancement than Ir-192	Lung cell line (A549) and prostate (DU145),
2017	Geraldo et al. (Geraldo et al., 2017) (28609167)	192Ir	Radiobiology	RB in vitro	Cytotoxicity, Cell Cycle Dynamics	HDR induces G2/M arrest, decreases proliferation	Human squamous cells carcinoma (A431)

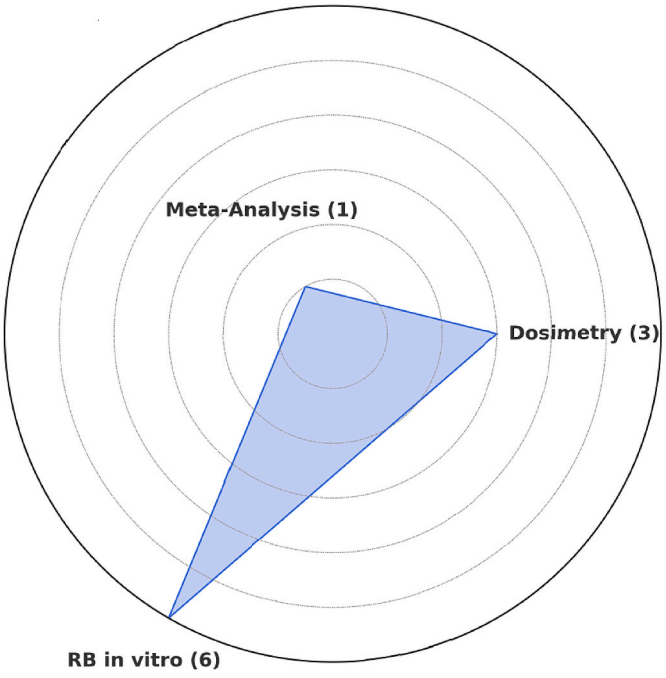

Fig. 1. Distribution of research metodology in *in vitro* HDR-IRT studies.

and MDA-MB-231), as well as normal fibroblasts (NIH/3T3). The study demonstrated that the combined treatment significantly enhanced radiosensitization, achieving a sensitiser enhancement ratio (SER) of 4.29 in MCF-7 cells, while maintaining high cell viability in control conditions, thus confirming the absence of intrinsic cytotoxicity from BiONPs and cisplatin at the administered doses (Talik et al., 2019). Shahhoseini et al. compared the cytotoxic effects of gold nanoparticles (AuNPs) in combination with two radiation sources, 50 kV eBx™ and Ir-192, on A549 lung and DU145 prostate cancer cells. Their findings revealed a stronger radiosensitizing effect with the low-energy eBx™ system, particularly in DU145 cells, where a dose enhancement factor (DEF) of 2.9 was observed, underscoring the influence of beam energy on nanoparticle-mediated effects (Shahhoseini et al., 2018). In another study the authors, explored radiation-induced bystander effects (RIBE) using a medium transfer approach, where supernatant from MCF-7 cells irradiated with Ir-192 HDR was transferred to non-irradiated MCF-7 and hFOB osteoblast cells. The results showed a significant decrease in cell viability and increased reactive oxygen species (ROS) production in both target and bystander cells, suggesting that HDR irradiation can trigger indirect cytotoxic mechanisms extending beyond the irradiated field (Bystander Effect Induced in Breast, 2020). Geraldo et al. focused on the cellular response of radioresistant A431 squamous carcinoma cells to fractionated HDR IRT (total dose 11 Gy). The treatment induced a marked reduction in proliferation and clonogenic survival, accompanied by a notable increase in G_2/M cell cycle arrest (Geraldo et al., 2017).

The α/β ratio was examined in three studies, either to characterize radiosensitivity or to develop predictive models for EQD2 estimation under varying conditions. One study conducted a comprehensive meta-analysis of α/β values derived from in vitro cervical cancer cell lines, revealing substantial variability across the literature, with a most probable value around 4.3 Gy. These findings suggest that the commonly adopted value of 10 Gy for most cancers and cervical cancer may not be appropriate for all cell types, as such discrepancies can lead to EQD2 uncertainties of clinical relevance. Additionally, the analysis found the variations of a/b values varied.

With radiation modalities and dose rate (Thayer-Freeman et al., 2025). Another study introduced a dedicated in vitro system (BAIRDA) using clinical Ir-192 HDR and PDR after loaders to quantify radiobiological parameters under realistic conditions. The α/β ratios measured across several cervical squamous cell carcinoma lines were consistently lower than standard assumptions, and the halftime of repair T1/2 larger than the conventionally used value of 1.5 h. These differences translated

into variations in EQD2 estimates of up to 27 Gy, reinforcing the importance of experimental validation in brachytherapy dose modeling (Chow et al., 2022). Complementing these efforts, a third study proposed a unified multi-activation (UMA) model capable of fitting survival curves over the full dose range, from low to high doses. This model offered improved agreement with experimental data compared to the traditional linear-quadratic (LQ) model and provided a dose-independent method to derive α/β and EQD2 values, which proved especially useful in the context of hypofractionated treatments such as HDR brachytherapy (Li et al., 2021).

Dosimetric assessments were also frequently conducted, particularly using Monte Carlo simulations or experimental validation with radiochromic films. These efforts aimed to quantify dose distribution and identify discrepancies between planned and absorbed doses, often leading to the definition of correction factors. These insights carry significant implications for the interpretations drawn from laboratory experiments, facilitating a more accurate analysis of interactions at the nanoscale level with cell dimensions. The computational experiments elucidated various parameters and their potential influence on the outcomes observed in *in vitro* studies using cell lines. Although these simulations represent only a preliminary step in understanding the complex network of interactions and effects that govern cellular responses, the results consistently revealed discrepancies between planned and measured doses in each cell culture plate. These differences were mainly attributed to the use of non-water-equivalent materials and the lack of full scattering contributions from the top of the irradiation platform. One study introduced an optimized in vitro setup for HDR brachytherapy using cell culture flasks and multiwell plates, demonstrating that differences of up to 7 % between planned and measured doses could be attributed to the physical configuration of the irradiation setup. The authors established individual dose correction factors to compensate for these discrepancies, emphasizing the need to standardize dosimetry in radiobiological research with HDR sources (Geraldo et al., 2021). Another work proposed a high-resolution voxelized Monte Carlo simulation framework to evaluate gold nanoparticle radiosensitization in a brachytherapy context. This study developed a predictive equation for the dose enhancement factor (DEF) as a function of AuNP concentration and validated it against published data. The results highlighted the energy dependence of DEF and the physical contribution of secondary electrons, thus supporting the model's use in designing nanoparticle-enhanced irradiation experiments (Araujo and Fonseca, 2025). A third study presented a modular, 3D-printed irradiation platform enabling the standardized delivery of HDR doses in vitro. Dosimetric evaluations using both Monte Carlo simulations and experimental validation with radiochromic films revealed measurable dose deviations caused by scattering inefficiencies and non-water-equivalent components, which were accounted for with empirical correction factors. The setup was proposed as a versatile tool for reproducible in vitro radiobiological experimentation across various culture formats (Silvus et al., 2024).

Some studies spanned multiple categories, contributing to the broader representation of research endpoints visualized in Fig. 2. Several methodological and conceptual trends become apparent across the selected studies. Radiobiological investigations remain the prevailing approach, reflecting the field's current emphasis on cellular and molecular endpoints. The widespread use of Ir-192 as the irradiation source mirrors clinical standards, and contributes to consistency across experimental settings. Although a range of biological endpoints was explored, such as viability, clonogenic survival, oxidative stress, and DNA damage, relatively few studies investigated radiobiological modeling or high-precision dosimetry. Furthermore, the methodological setup was largely homogeneous, as all studies employed standard 2D monolayer cultures, while only one integrated a custom 3D-printed platform to improve dose delivery accuracy. This lack of more advanced or physiologically relevant models, such as spheroids or organoids, underscores an opportunity for future studies to better

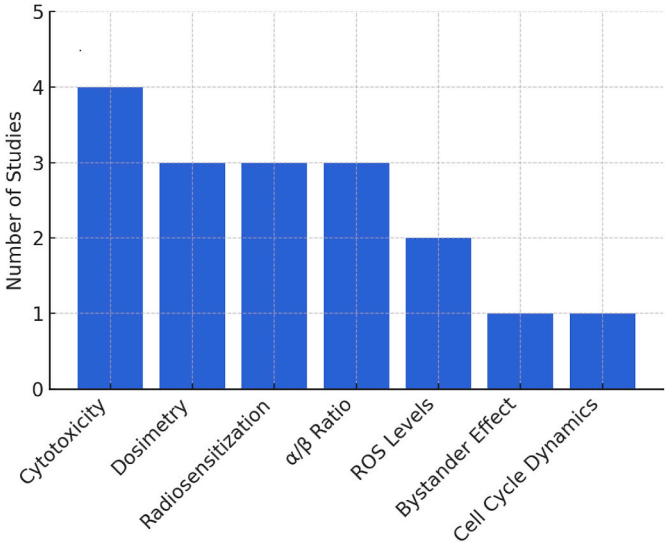

Fig. 2. Distribution of studied endpoints in *in vitro* HDR-IRT research.

replicate *in vivo*-like conditions. Together, these observations point to a need for a more integrated experimental framework, which combines biological, physical, and computational elements, to improve the translational value of in vitro HDR IRT research.

Two studies investigated reactive oxygen species (ROS) production to evaluate oxidative stress as a mechanism of radiation-induced damage, both employing clinically used high-dose-rate (HDR) brachytherapy with an Ir-192 source. In the first, the authors demonstrated that conditioned medium from MCF-7 breast cancer cells irradiated with HDR Ir-192 brachytherapy significantly reduced the viability and clonogenic survival of both non-irradiated MCF-7 and human osteoblast (hFOB 1.19) cells. This radiation-induced bystander effect (RIBE) was accompanied by a sustained increase in intracellular ROS levels, suggesting oxidative stress as a key mediator of the observed indirect cytotoxicity (Bystander Effect Induced in Breast, 2020). The second study evaluated the radiosensitization potential of bismuth oxide nanoparticles (BiONPs), administered alone and in combination with cisplatin, under HDR Ir-192 brachytherapy conditions. The results revealed a significant enhancement of radiation-induced cytotoxicity in MCF-7 cells, with the combination BiONPs–cisplatin achieving the highest radiosensitization effect. This effect was further supported by increased ROS generation in the treated groups, highlighting the synergistic potential of nanoparticle-based sensitizers in HDR brachytherapy (Talik et al., 2019). In a separate study, gold nanoparticles (AuNPs) were tested for their radiosensitizing effect under both conventional HDR Ir-192 brachytherapy and Xoft® Axxent® electronic brachytherapy (eBx™) using low-energy X-rays (~50 kV) (Shahhoseini et al., 2018).

Finally, the biological consequences of HDR-IRT were further explored through one study investigating cell cycle dynamics. The study, conducted on the A431 human squamous cell carcinoma cell line, characterized by its radioresistant behavior, demonstrated that exposure to two 11 Gy fractions of HDR brachytherapy using a clinical Ir-192 source led to a marked decrease in proliferation rate and clonogenic survival. Flow cytometry analysis revealed a significant accumulation of cells in the G2/M phase, indicating a radiation-induced cell cycle arrest at this checkpoint. Morphological observations showed nuclear shrinkage without evidence of classical apoptotic or necrotic processes, suggesting that mitotic catastrophe was the dominant mechanism of cell death. These findings underscore the potential of HDR IRT to impair cellular progression and induce non-apoptotic death pathways in resistant tumor phenotypes, contributing to improved therapeutic outcomes in challenging oncologic contexts (Geraldo et al., 2017).

In terms of methodology, all studies employed 2D monolayer cultures, typically using six-well or 96-well plates and T25 flasks. One study introduced a 3D-printed irradiation platform to enhance precision and standardization in dose delivery, reflecting growing technological innovation in experimental design (Silvus et al., 2024). Similarly, Geraldo et al. developed an acrylic-based platform for in vitro HDR brachytherapy that accommodates multiple commercial culture vessels and enables dose planning through clinical software and Monte Carlo simulations, ensuring uniform dose distribution and enabling the application of dose correction factors based on flask geometry and scattering conditions (Geraldo et al., 2021). Nonetheless, despite recent advances in technology, none of the reviewed studies employed 3D biological models such as spheroids or organoids, underscoring an opportunity for future research to adopt more physiologically relevant in vitro systems.

A diverse range of cell lines was employed across the selected studies, reflecting both tumor-specific investigations and the need for comparative radiobiological analyses. In total, at least thirteen human and two murine cell lines were used. Among the most frequently studied cancer models were MCF-7 (breast carcinoma), CaSki, SiHa, C-33A, and SW756 (cervical squamous and adenocarcinoma lines), A549 (lung carcinoma), DU145 (prostate carcinoma), and A431 (epidermoid squamous cell carcinoma). These were complemented in some studies by non-tumorigenic or reference cell lines, including hFOB 1.19 human osteoblasts and NIH/3T3 murine fibroblasts, which served as healthy comparators to assess differential radiation responses.

To enhance the reproducibility and downstream utility of this review, we systematically extracted detailed methodological and dosimetric information from each included HDR in vitro study. A comprehensive summary is provided in Supplementary Table S1, reporting for each study the HDR source, activity or dose rate at reference, source-to-sample geometry, fractionation scheme, planned versus delivered dose, and dose calculation formalism (TG-43 vs model-based algorithms). For studies performing Monte Carlo simulations, we also reported the code, version, cross-section libraries, physics lists, and voxel sizes when available. In addition, the table specifies whether independent dosimetric validation was performed, indicating the measurement technique (radiochromic film or ion chamber) and associated uncertainties.

A quantitative summary of the main methodological features of the included studies highlights significant variability across experimental designs. Among the ten studies analyzed, a minority (3; 30 %) performed Monte Carlo-based dosimetry, and only three (30 %) reported independent dosimetric validation using radiochromic films or ion chambers. Regarding biological endpoints, cytotoxicity assays were the most frequently used (5/10 studies; 50 %), followed by clonogenic survival (4/10; 40 %) and α/β estimation (3/10; 30 %). Only two studies (20 %) assessed reactive oxygen species (ROS) and bystander effects, while none investigated immune-related markers or adopted physiologically relevant 3D culture models. This distribution highlights the limited diversity of explored endpoints and underscores the need for more comprehensive HDR in vitro investigations integrating advanced biological models.

4. Discussion

The findings of this literature review highlight a limitation in the breadth and depth of in vitro research on HDR-IRT, emphasizing the need for a more extensive preclinical research effort in this area. Despite its established role in clinical oncology, HDR-IRT remains underrepresented in preclinical RB, with only a small number of in vitro studies published over the past decade. Specifically, our search identified only 10 in vitro studies using HDR sources, compared to 78 studies employing LDR sources, further underscoring the disproportionate representation of LDR in the existing literature and the need for HDR-specific experimental models.

An additional limitation of this review lies in the small number of identified HDR in vitro studies ($n = 10$), which inherently restricts the strength and generalizability of our conclusions. While this limited evidence base highlights an important research gap, it also constrains the possibility of drawing statistically robust inferences and reduces the consistency of endpoint stratification. To better understand the reliability of the available evidence, we performed a brief quality and risk-of-bias appraisal tailored to in vitro HDR research. Among the ten included studies, only a minority (Stewart et al., 2025) reported independent dosimetric validation using radiochromic films or ion chamber measurements, whereas four relied exclusively on Monte Carlo simulations and three did not provide sufficient details to assess the accuracy of dose delivery. Moreover, only a minority (Stewart et al., 2025) explicitly linked biological endpoints such as clonogenic survival or ROS production to experimentally verified dose distributions, while most studies (Tubin et al., 2023) derived biological findings from nominal planned doses without accounting for potential heterogeneities or measurement uncertainties. This lack of standardized verification introduces additional variability, especially given that some studies have reported dose deviations of up to 7 % due to platform geometry and scattering inefficiencies. Together, these findings underscore the need for harmonized methodologies integrating robust dosimetric verification and systematic reporting of experimental conditions to strengthen the reproducibility and translational value of future HDR in vitro studies.

The predominance of radiobiological investigations over dosimetric studies suggests a strong interest in understanding cellular responses to HDR-IRT, which is essential for translational applications. Most of these studies rely on two-dimensional (2D) cell cultures, which offer simplicity and reproducibility but fall short in recapitulating the complex architecture and microenvironmental features of *in vivo* tumors. While Dosimetric assessments using Monte Carlo simulations and radiochromic film measurements were reported in several studies, revealing dose heterogeneities and deviations from planned values due to set up geometry and material limitations. These findings are informative, the integration of precise dosimetric data with biologically relevant models remains insufficient. Advanced experimental set ups, such as 3D-printed irradiation platforms, have been introduced in isolated cases but are yet to be widely adopted or combined with complex cell culture systems. The increasing availability and accessibility of 3D printing technologies offer a valuable opportunity to enhance the reproducibility and precision of experimental setups in HDR-IRT research. Specifically, customized 3D-printed platforms enable the reliable positioning of individual catheters, even in in vitro irradiation settings where standard clinical applicators are not used. This approach allows for more accurate dose delivery and better control over geometrical parameters, facilitating the standardization of preclinical experiments and improving their translational relevance.

The choice of IRT over EBRT is primarily guided by its advantages in dose delivery and spatial distribution rather than differences in intrinsic radiobiological properties. This localized dose deposition leverages the volume effect in normal tissues and often results in lower dose rates to non-target areas, providing an additional therapeutic advantage by minimizing normal tissue toxicity. These advantages are particularly relevant in anatomical sites where achieving a conformal dose distribution with EBRT is dosimetrically challenging, such as in skin and head and neck tumors. However, the non-uniform radiation field generated around the implanted source introduces complex radiobiological dynamics that require further investigation to fully understand their therapeutic implications (Placidi et al., 2023, 2024). In close proximity to the source, HDRs lead to substantial cell killing, whereas in peripheral regions, lower dose rates result in reduced biological effects. This creates a steep gradient in cellular response, ranging from complete cell inactivation to survival even of radiosensitive populations. Given these distinctive features, particularly the spatial and temporal heterogeneity in dose rate and its influence on tissue response, it is essential to investigate the underlying radiation-induced biological mechanisms at

the cellular level in a controlled in vitro environment. Such studies are fundamental to improving our understanding and optimizing therapeutic outcomes, especially in the context of HDR-IRT, which is characterized by the delivery of high radiation dose rates localized to small, well-defined target volumes [Joiner 2025].

Among RB-focused studies, a variety of biological endpoints have been investigated, yet their distribution remains uneven. Cytotoxicity assessment is the most frequently examined parameter, highlighting the widespread reliance on cell viability assays as a primary measure of radiation-induced damage. However, while cytotoxicity provides essential insights into cell survival, it does not capture the broader spectrum of radiation effects, including DNA damage repair mechanisms, sublethal damage accumulation, and long-term survival potential (Annede et al., 2020).

Similarly, α/β ratio assessments, primarily used to evaluate radiosensitivity or develop predictive models, remain a cornerstone in radiobiological research. However, despite their importance, there is a lack of studies applying α/β ratio analysis to novel HDR-IRT fractionation schedules, which could provide valuable insights into optimizing treatment protocols. Radiosensitization, a promising strategy for enhancing therapeutic efficacy, has been addressed in only a handful of studies, primarily through the use of nanoparticles, and remains an underexplored area in HDR-IRT research, despite its potential clinical impact (Joiner).

The role of oxidative stress in radiation response is widely recognized, yet ROS measurements were reported in only a few studies. As ROS levels influence DNA damage, apoptosis induction, and treatment resistance, integrating more sophisticated oxidative stress assays could help refine our understanding of how HDR-IRT affects tumor biology (ref. PMID 40377005). Additionally, the immune response to radiation exposure is an emerging area of investigation, with ROS measurements serving as an initial indicator of oxidative stress-mediated immune activation. ROS play a key role in shaping the radiation-induced immune response by influencing inflammatory signaling, cytokine release, and the activation of immune-modulating pathways. However, their direct contribution to immunogenic effects in HDR-IRT remains largely unexplored (Zheng et al., 2023).

In addition to direct cellular responses, the bystander effect represents another indirect mechanism influencing tumor response to HDR-IRT. This phenomenon, in which non-irradiated cells exhibit radiation-like responses due to signals from irradiated neighbors, remains underrepresented in HDR-IRT research. Understanding how bystander effects contribute to tumor control or resistance may provide valuable insights into potential systemic effects of localized irradiation. Investigating these indirect radiation effects could further clarify how HDR-IRT influences the tumor microenvironment beyond direct DNA damage.

Beyond biological endpoints, methodological limitations also emerge in the current body of literature. The exclusive use of 2D in vitro models restricts the ability to accurately replicate the tumor microenvironment, limiting the translational potential of these studies. While monolayer cultures remain a standard in preclinical RB due to their simplicity and reproducibility, they fail to capture critical tumor characteristics, such as hypoxia gradients, extracellular matrix interactions, and heterogeneous cellular populations. Despite the increasing use of 3D cell culture models, including spheroids and organoids, which are increasingly recognized as essential tools for mimicking tumor physiology; none of the analyzed studies incorporated these more advanced systems (Guan and Huang, 2022). The lack of 3D systems represents a substantial gap, limiting the translational potential of preclinical HDR-IRT research.

In addition to these scientific and experimental gaps, some methodological limitations of this review should be acknowledged. The search was restricted to English-language publications and relied on a focused set of search terms, which may have led to the omission of relevant studies. Furthermore, PubMed and Scopus were selected as primary databases because of their comprehensive coverage of clinical and preclinical HDR-IRT literature.

This review provides a practical reference for researchers aiming to design or replicate HDR in vitro experiments. The heterogeneity observed in HDR source parameters, dosimetric formalisms, Monte Carlo implementation, and independent dose verification methods underscore the lack of standardized methodologies in this field.

5. Future perspectives

To advance the preclinical study of HDR-IRT, future research must aim to bridge the remaining translational gaps by adopting interdisciplinary approaches that integrate RB, engineering, and computational modeling. Rather than simply refining existing 2D protocols, the field should prioritize the development of complex experimental frameworks that reflect the multifaceted and heterogeneous nature of tumor response to HDR-IRT.

Given the heterogeneous nature of the tumor microenvironment, both in terms of cellular composition and biological response, it is essential to move beyond monoculture models. Tumor tissues often comprise not only cancer cells but also a variety of stromal, endothelial, and immune cells, all of which contribute to treatment resistance and therapeutic outcome. In this context, implementing co-culture systems involving different cell types, such as malignant and non-malignant epithelial cells, fibroblasts, or immune cells, may offer deeper insight into the cellular crosstalk that occurs during and after interventional radiotherapy. Studying how these cell populations interact when exposed to the same radiation stress could help elucidate the mechanisms of damage propagation, survival signaling, and microenvironmental remodeling (Kunz-Schughart et al., 2004).

In particular, modeling scenarios where healthy and tumor tissues are in direct contact, as frequently occurs in clinical HDR-IRT applications, could shed light on how differential responses to localized high-dose exposure evolve over time. Such approaches could contribute to a more comprehensive understanding of how radiation impacts the interface between tumor and normal tissue, providing a foundation for strategies that selectively enhance tumor control while preserving surrounding healthy structures.

Looking ahead, future HDR in vitro studies should integrate physiologically relevant 3D models, such as spheroids, organoids, and co-cultures, with validated 3D dosimetry combining Monte Carlo-based dose mapping and experimental verification. This approach would improve reproducibility, enhance biological relevance, and increase the translational impact of HDR radiobiology research.

A notable technological advancement observed in recent HDR-IRT research was the introduction of a 3D-printed irradiation platform, designed to improve dosimetric precision and standardize irradiation procedures in vitro. While this innovation represents a step forward, it remains insufficient on its own to overcome the limitations of traditional monolayer cultures. A more impactful direction lies in the development of biomimetic platforms that integrate three-dimensional (3D) cellular architectures with advanced dosimetric systems. These systems, especially when equipped with real-time dose monitoring and modular designs, allow for dynamic assessment of dose-response relationships, DNA repair kinetics, and radiation-induced signaling within biologically relevant environments (Silvus et al., 2024; Pinto et al., 2020).

Future HDR-IRT studies should therefore prioritize the integration of these advanced 3D models with precise and scalable dosimetric techniques, including high-throughput screening systems and real-time monitoring technologies. This combined approach has the potential to significantly enhance the predictive accuracy of in vitro experiments and to better replicate the complexity of *in vivo* tumor microenvironments (Antonelli, 2023).

Taken together, these advancements call for a paradigm shift in HDR-IRT preclinical research. Embracing 3D culture systems, broadening biological endpoints to include DNA repair mechanisms, immune activation, and oxidative stress pathways, and implementing

multimodal methodologies will substantially strengthen the translational value of in vitro models. Bridging these gaps will ultimately support the development of more effective and personalized HDR-IRT protocols (Nag, 2004).

Furthermore, expanding the range of biological endpoints is essential to fully capture the complexity of tumor response to HDR-IRT. While cytotoxicity and clonogenic survival remain fundamental measures, there is growing recognition of the importance of additional processes such as immunogenic modulation, oxidative stress pathways, and metabolic rewiring. Exploring these dimensions could help identify novel therapeutic opportunities and inform the development of combined modality approaches that enhance treatment efficacy (Zheng et al., 2023).

Among these, the immune response is increasingly regarded as a critical component of radiotherapy outcomes, particularly when integrated with immunotherapy. Gaining a deeper understanding of the mechanisms that regulate immune activation and suppression, including cytokine signaling, antigen presentation, and the expression of immune checkpoint molecules, could help close important knowledge gaps in the field of radiation-induced immunogenicity. This knowledge may also guide strategies to enhance tumor immunogenicity or mitigate immune evasion, ultimately improving therapeutic success (Demaria et al., 2015).

Nonetheless, it remains crucial to maintain a strong focus on cytotoxic endpoints, particularly given their close association with cellular radiosensitivity. Radiosensitivity varies substantially across cell types and tumor subtypes, and characterizing these differences can significantly influence clinical decision-making. By understanding how specific tumor and normal tissue cells respond not only in terms of survival but also in their interaction with the immune system, it becomes possible to guide treatment selection more effectively.

Importantly, tailoring radiotherapy based on tumor-specific radiosensitivity could improve both local tumor control and patient safety. Personalized treatment strategies that integrate radiosensitivity profiles can allow for the adjustment of dose and fractionation, potentially reducing unnecessary radiation exposure to surrounding organs at risk. This approach may help minimize treatment-related toxicity while maintaining, or even enhancing, therapeutic efficacy (Joiner; Reits et al., 2006; Kerns et al., 2014).

In addition, emerging evidence suggests that HDR-IRT may differentially affect tumor and stromal compartments. This spatial selectivity could have profound implications for tumor progression, angiogenesis, and immune infiltration. Understanding these differential effects will be critical to advancing personalized radiotherapy approaches. To this end, the adoption of multicellular and co-culture systems, capable of recapitulating the interactions between cancer cells and their surrounding microenvironment, may substantially enhance the predictive power of in vitro models and offer new insights into how the tumor and healthy tissue interface responds to HDR-induced stress (Nag, 2004).

Ultimately, future studies should adopt a system-level perspective, integrating biological, physical, and technical dimensions of HDR-IRT to generate comprehensive, clinically actionable insights. This approach will support the evolution of in vitro models from static testing platforms to dynamic research tools capable of informing protocol development and precision medicine applications.

6. Conclusions

This review and gap analysis reveals a substantial translational limitation in current in vitro HDR-IRT research, primarily due to the continued reliance on oversimplified 2D culture systems that inadequately recapitulate the spatial and biological complexity of the clinical tumor microenvironment. Critical radiobiological mechanisms remain insufficiently characterized, and the lack of standardized, high-precision dosimetric approaches further compromises reproducibility and clinical relevance. The integration of physiologically relevant 3D culture models, along with the implementation of tailored 3D-printed irradiation platforms and advanced dosimetric strategies, is essential to reduce the methodological gap between preclinical experimentation and clinical application. Addressing these limitations will enable more robust in vitro modeling, ultimately supporting the development of effective, individualized HDR-IRT therapies.

CRediT authorship contribution statement

Enrico Rosa: Writing – original draft, Data curation, Conceptualization. **Benedetta Niccolini:** Writing – original draft, Data curation, Conceptualization. **Riccardo Di Santo:** Visualization, Validation, Methodology. **Bruno Fionda:** Validation, Formal analysis, Data curation. **Maria Vaccaro:** Writing – original draft, Visualization. **Elisa Placidi:** Visualization, Supervision. **Elisabetta Tabolacci:** Writing – review & editing, Visualization, Validation, Supervision. **Marco De Spirito:** Visualization, Supervision, Project administration. **Monica Mangoni:** Writing – review & editing, Visualization, Validation. **Luca Tagliaferri:** Validation, Supervision, Project administration. **Gabriele Ciasca:** Validation, Supervision, Project administration, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apradiso.2025.112284.

Data availability

No data was used for the research described in the article.

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