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Perspective

Perspective to clinical translation of radiotherapy-activated prodrugs: challenges and advances

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Cancer remains one of the foremost causes of mortality worldwide, persisting despite significant advances in therapeutic methodologies.[1] The current landscape of cancer treatment is defined by three primary modalities: surgery, chemotherapy, and radiotherapy. Over the past few decades, chemotherapy has evolved from traditional cytotoxic drugs to more sophisticated targeted therapies, including tyrosine kinase inhibitors and monoclonal antibodies, which disrupt specific molecular pathways.[2] However, the efficacy of monotherapy is often limited in cases of locally advanced or metastatic patients, largely due to tumor heterogeneity, which undermines the effectiveness of single-agent treatments. As a result, a combination with adjuvant or neoadjuvant therapies is often necessary to improve outcomes.

Radiotherapy technological advancements, such as intensity-modulated radiotherapy, stereotactic body radiotherapy (SBRT), adaptive radiotherapy, proton beam therapy, heavy ion radiotherapy, microbeam radiotherapy, and FLASH-radiotherapy have further optimized radiotherapy's precision, effectiveness, and safety.[3] Nonetheless, radiotherapy's clinical applicability is still limited by challenges inherent to radiation, including toxicity to surrounding healthy tissues and limited efficacy against radioresistant tumors. Similar to single-agent chemotherapy, radiotherapy is also constrained by tumor heterogeneity, encompassing factors such as variability in individual patient and tissue tolerance to radiation, differences in tumor distribution, and cumulative radiation doses across multiple sessions.

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These limitations collectively undermine the effectiveness and safety of radiotherapy in specific clinical contexts, ultimately leading to recurrence and metastasis in a significant proportion of patients. These limitations underscore the pressing need for adjunctive therapies that expand radiotherapy's therapeutic reach, all while minimizing adverse effects on surrounding healthy tissues.

Combining modalities—such as adjuvant, neoadjuvant, concurrent chemoradiotherapy, or immunotherapy sequential radiotherapy—have shown synergistic benefits in numerous studies, leading to improved survival rates and reduced recurrence across multiple cancer types. According to the 2022 epidemiological data on the top 12 malignancies in China released by the National Cancer Center and the latest clinical treatment guidelines, combined chemoradiotherapy is a key recommended approach in the guidelines for 10 of these cancers, excluding breast and prostate cancers. It has become a cornerstone in the treatment of various solid tumors, particularly locally advanced nasopharyngeal and cervical cancers, and is established as the standard of care for preoperative management of locally advanced rectal cancer (Supplementary Text 1 online). However, combining modalities also encounter challenges such as stacked toxicity and decreased patient adherence, especially hematologic toxicity, and increases both acute and chronic radiation-induced side effects, particularly in patients with poor performance status. This clinical demand underscores the importance and urgency of developing new combination therapy technologies with "toxicity reduction and efficacy enhancement".

Radiotherapy-activated prodrugs (RAPs) are specifically engineered to remain inactive until exposed to ionizing radiation, at which point they are activated therapeutically within the irradiated tumor site (Fig. 1). This selective activation provides dual benefits: enhancing the localized chemotherapy effects of radiotherapy region while sparing non-target tissues from systemic toxicity. By harnessing the spatiotemporal precision of radiotherapy technologies, RAPs have the potential to improve the therapeutic index of chemotherapy and address chemoresistance in certain tumor types. For instance, platinum-based RAPs, which incorporate radiation-activated linkers, can deliver potent DNA-damaging effects

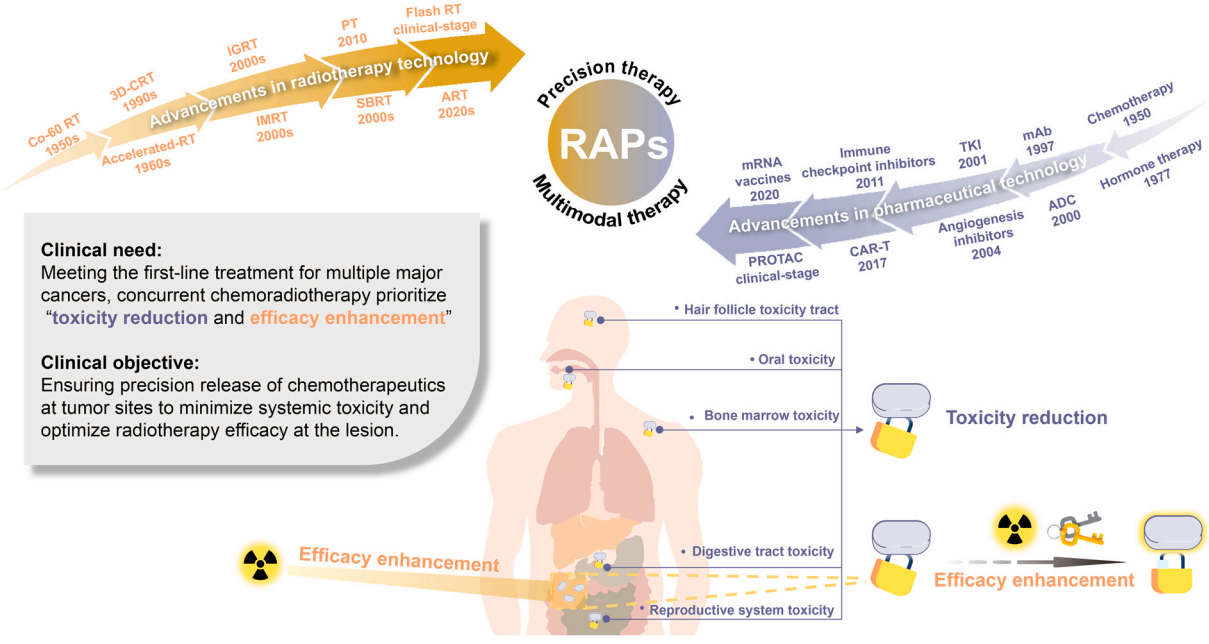

Fig. 1. Advances in radiotherapy technology and the development of anti-tumor drugs have led to the generation of radiotherapy-activated prodrugs (RAPs). The timeline in the figure represents key milestones in the development of these therapies with years corresponding to the market launches. 3D-CRT: 3D conformal radiation therapy; Accelerated-RT: accelerated radiation therapy; ADC: antibody-drug conjugate; ART: adaptive radiation therapy; CAR-T: Chimeric antigen receptor T-cell therapy; Co-60 RT: cobalt-60 radiation therapy; Flash RT: flash radiation therapy; IGRT: image-guided radiation therapy; IMRT: intensity-modulated radiation therapy; mAb: monoclonal antibody; PROTAC: proteolysis-targeting chimeras; PT: proton therapy; SBRT: stereotactic body radiation therapy; TKI: tyrosine kinase inhibitor.

within the tumor microenvironment upon radiation activation.[4] This approach circumvents the limitations of conventional chemotherapy, which often distributes cytotoxic agents systemically, leading to off-target effects. The versatility of RAPs aligns with the principles of precision oncology, where therapies are increasingly tailored to the molecular and cellular characteristics of each patient's tumor.

RAPs' compatibility with existing radiotherapy protocols makes them highly practical for clinical integration, providing oncologists with a powerful tool to enhance local tumor control without increasing systemic toxicity. In line with the broader movement toward safer, more targeted cancer therapies, RAPs represent an adaptable and effective modality that could redefine the role of radiotherapy in cancer treatment, expanding its applicability and improving outcomes for patients with difficult-to-treat cancers. In 2020 and 2021, our team and Bradley's team have independently made significant strides in this field, developing innovative methods for enhancing *in vivo* tumor-specific drug (monomethyl auristatin E, doxorubicin, etc.) activation via irradiation.[5,6] Since the pioneering study of RAPs, our team has made notable progress by creating N-oxide-based prodrugs in 2022, which utilize radiotherapy-induced hydrated electrons to selectively activate drugs within the tumor microenvironment.[7] This approach represented a breakthrough in minimizing off-target effects while increasing efficacy, particularly in regions where conventional therapies are often limited. The team further expanded RAP applications by incorporating quaternary ammonium groups as cleavable elements within prodrugs, enabling the targeted release of drugs in response to hydrated electrons.[8] This method was especially effective in hypoxic tumor regions, which tend to be resistant to standard treatments, broadening the potential of radiotherapy-combination therapies.

Continuously seeking breakthroughs and innovation, our team introduced N-alkyl-4-picolinium as a novel radiation-cleavable protecting group in 2024, significantly enhancing the stability and activation precision of antibody-drug conjugate.[9] This advancement underscores the potential of radiation-triggered groups for improving the safety and efficacy of chemotherapeutic applications. We also adapted the radiotherapy-triggered reduction strategy to platinum (IV) complexes, effectively converting them to cytotoxic platinum (II) at the tumor site. This targeted activation enhances therapeutic effects while minimizing systemic toxicity, representing a substantial improvement in integrating platinum-based chemotherapy with radiotherapy. Most recently, the team combined diagnostic imaging with therapeutic activation using the radiopharmaceutical ^{18}F-fluorodeoxyglucose (FDG) to trigger prodrug activation within tumors.[10] This innovation allows for real-time monitoring of therapeutic responses while enabling precise, tumor-specific drug release, representing a significant advancement in precision medicine through a combined diagnostic-therapeutic platform. Together, these contributions highlight the pioneering efforts in refining RAPs and setting new standards in targeted cancer therapy.

Above all, the field of RAPs has seen considerable advancements, with several landmark studies (Supplementary Text 2 online) from other research groups reflecting the growing global interest in this technology. In 2022, Yang et al.[11] developed a radiotherapy-triggered PROTAC, enabling precise, spatiotemporal protein degradation with radiation—a technique that is particularly advantageous in minimizing systemic toxicity often associated with PROTACs. In 2023, Xu et al.[12] reported a metal-organic framework containing an X-ray-triggerable prodrug, achieving a synergistic effect in chemoradiotherapy through enhanced local drug release in response to radiotherapy-induced reactive oxygen species. In 2024, Ogawara et al.[13] designed X-ray-triggerable azo compounds with improved efficiency for hydrated electron reaction, demonstrating the potential of caged compounds for deep-tissue drug activation using high-energy X-rays. Liu et al.[14] further expanded the RAP toolkit by exploring the oxidative capabilities of organochlorides under low-dose ionizing radiation, using peroxyl radicals to regulate drug release. Quintana et al.[15] advanced a radiation-activated albumin-bound and antibody prodrug system with extended pharmacokinetics, achieving enhanced site-specific activation with minimal off-target effects, guided by computational modeling. Collectively, these studies contributed to the field's rapid development and a shared commitment to optimizing RAPs for targeted cancer therapy. This growing body of research highlights the broad applicability of the RAP approach, demonstrating the potential to impact diverse areas within precision oncology and offering promising avenues for continued innovation.

Harnessing the primary advantages of RAPs for clinical translation as a novel technology. RAPs present a novel strategy that combines the clinical benefits of radiotherapy while lifting the restriction on

insufficient efficacy and safety. Integrating RAPs into radiotherapy regimens holds substantial promise for enhancing clinical outcomes. RAPs align closely with the principles of precision medicine, allowing treatments to be tailored to individual tumor characteristics and providing a more personalized approach to cancer care.

Selecting appropriate indications for RAPs is a strategic process aimed at maximizing clinical efficacy and addressing current treatment gaps. First, indication selection prioritizes cancers in which chemoradiotherapy is already a well-established standard protocol. In these cases, introducing RAPs can enhance therapeutic outcomes without significantly altering existing treatment protocols, making RAPs an easily integrated enhancement to current regimens. Examples of priority cancers include colorectal cancer, nasopharyngeal carcinoma, head and neck cancers, esophageal cancer, cervical cancer, hepatocellular carcinoma, and locally advanced lung cancer. A second key criterion is dual sensitivity—tumors highly responsive to both radiotherapy and chemotherapy present an optimal opportunity for RAP application, as RAPs can synergize with radiotherapy to maximize synergistic antitumor effects while minimizing toxicity. In the future, RAPs are also expected to be applied to indications for radiotherapy resistance or chemotherapy resistance. Radiotherapy regimens for colorectal cancer are typically employed in preoperative, postoperative, or palliative contexts, with variations in fractionation dose and total treatment duration depending on individual patient requirements.

Standard fractionation often involves daily doses of 1.8–2 Gy over 5–6 weeks, reaching a cumulative dose of approximately 50–60 Gy. Alternatively, accelerated approaches, such as hypofractionated schedules (6 Gy $\times$ 5 fractions), deliver larger doses per fraction over shorter periods, often completed within one to two weeks. SBRT is also employed in select cases benefiting patients with inoperable or recurrent tumors by achieving local control in a shorter timeframe.[16] Chemotherapy for colorectal cancer, particularly in advanced stages, largely depends on platinum-based drugs such as oxaliplatin, typically administered with fluorouracil (5-FU) and leucovorin in the FOLFOX regimen. Another commonly employed regimen is XELOX, which combines capecitabine with oxaliplatin.[17] Due to the toxicity of chemotherapy drugs, patients require a certain period of recovery interval. Standard treatment cycles may extend up to 12 cycles, covering several months. The extended duration and toxicity of chemotherapy require meticulous management, often creating scheduling conflicts with radiotherapy, particularly for patients receiving concurrent treatments. Therefore, in the clinical practice of RAPs, it is necessary to formulate an appropriate treatment plan through dose escalation trials while ensuring patient tolerance of toxicity.

Integrating RAPs into cancer treatment regimens faces unique challenges and opportunities. A primary issue lies in the mismatch between radiotherapy and chemotherapy cycles, as radiotherapy for colorectal cancer often concludes in approximately one month, whereas chemotherapy typically extends over a significantly longer duration, often lasting several months. However, the dosing regimen and duration of clinical radiotherapy cycles directly impact the optimal therapeutic outcomes of RAPs. To address these issues, our proposed RAP-based approach synchronizes radiotherapy and chemotherapy effects within the radiotherapy treatment period, enabling a dual-action therapeutic benefit. RAPs strategy achieves higher localized drug concentrations with minimized systemic toxicity, aligning chemotherapy effects with the radiotherapy schedule. By consolidating both treatments into the radiotherapy timeframe, RAPs could reduce the chemotherapy cycles, improve patient adherence, and alleviate the treatment burden. Simultaneously, it may provide new options for adjuvant therapy and fight for surgical opportunities for patients. This integrated approach maximizes the tumoricidal effects of both modalities within a streamlined schedule and enhances patient tolerability by reducing cumulative side effects and eliminating the logistical challenges associated with extended dual therapies.

Preclinical pharmacodynamic (PD)/pharmacokinetic (PK)/toxicity study of RAPs. Beyond the foundational tasks of selecting appropriate clinical indications and harmonizing radiotherapy and drug dosage schedules, additional considerations center on the PK and PD properties of the prodrug, both pre- and post-activation by radiotherapy, including establishing modeling of PK/PD relationships of RAPs. Unlike traditional prodrugs activated by intracellular enzymes *in vivo*, prodrugs activated by radiotherapy have unique PK characteristics. The amount of active ingredient released also depends on the dose of radiotherapy, which will prompt us to study the correlation between radiation dose and drug dose. Therefore, the PK and tissue distribution of prodrug before and after radiotherapy activation need to be

further studied. Radionuclide therapy is another way to activate RAP. According to Guo et al.,[18] targeted radionuclide drugs can specifically activate RAPs at the tumor site to control distant metastasis. The PK of radionuclide-activated RAPs is also very unique. Targeted radionuclide therapy activates RAPs requiring a comprehensive insight into the pharmacokinetics of both the radionuclide drug and RAPs. Theoretically, radiopharmaceuticals should be enriched to the tumor site before RAP injection, and a large absorbed dose of radiation at the tumor site can activate RAPs in a high concentration. These PK/PD characteristics are crucial in guiding Phase I trial design, typically involving human dose-escalation studies. Effective trial design requires a prospective translational strategy to bridge preclinical findings with clinical applicability, including the meticulous selection of animal models that accurately reflect human dose-response and PK profiles for RAPs. As for PD, it is necessary to clarify the mechanism of action of the RAPs *in vitro* experiments, including drug release efficiency, cell proliferation, and colony-forming assay. The therapeutic effect should be confirmed in the xenograft tumor model, distal metastasis mouse model, patient-derived tumor xenograft model, or large animal tumor model. Developing a robust preclinical model that reliably predicts human PK/PD outcomes is essential, as these models must specifically account for prodrug activation within irradiated tissues. This unique aspect is more complicated than preclinical protocol compared to conventional small molecule drugs. In addition to PK/PD, toxicology and safety pharmacology will also be an essential part of investigating RAPs. Proving their stability and safety under non-irradiation conditions is the primary prerequisite for conducting clinical trials. Therefore, understanding the interplay between dosing regimens, PK/PD dynamics, and toxicological parameters in preclinical studies is essential for determining optimal dose selection and safety for human trials. Good Laboratory Practice-compliant toxicity studies are equally important, providing data on RAP-specific toxicities and ensuring that prodrug activation within irradiated tissues does not produce unforeseen adverse effects. The metabolites produced after irradiation should also be given sufficient research. Consequently, these animal studies must account for comprehensive exposure scenarios, ideally in models that approximate human metabolic and physiological responses.

Chemistry, manufacturing, and control (CMC) process of RAP. Moreover, the CMC processes for RAP development are critical, given the pivotal role in stringent quality control throughout the prodrug lifecycle. Unlike traditional chemotherapeutics, RAPs necessitate meticulous control over impurities in both the prodrug and the activated compounds generated post-radiotherapy. Key considerations in the CMC process include the development of strategies to maintain the prodrug's stability, thereby preventing unintended premature activation of harmful degradation products during storage or administration. This is critical to preserving the therapeutic efficacy and safety of the RAPs. Furthermore, any products resulting from radiotherapy activation must be thoroughly analyzed to confirm the absence of harmful degradation by-products that could pose risks to patient safety. Analytical techniques capable of precisely tracking these parameters are essential to support both safety evaluations and regulatory submissions, ensuring compliance with stringent standards. An essential aspect of the CMC process involves simulating the prodrug's release behavior under radiotherapy conditions. The RAPs released after irradiation can be simulated by the doping method during CMC, where controlled experiments are conducted to mimic the irradiation process. These simulations provide critical data on the RAP's release kinetics, chemical transformations, and stability profiles. To support these stringent requirements, advanced analytical techniques must be employed. These techniques can accurately track key parameters, including impurity levels, degradation pathways, and activation efficiency. The generated data underpins the safety and efficacy evaluations required for clinical progression and forms the basis for comprehensive regulatory submissions. Adhering to these high standards ensures compliance with global regulatory frameworks and fosters confidence in the reliability of RAPs as a therapeutic modality.

RAP multidisciplinary cooperation. The inherently interdisciplinary nature of RAP development underscores the need for collaborative expertise. Close collaboration between medicinal chemists, clinical radiologists, oncologists, pharmacologists, and regulatory specialists is essential for navigating the intricate requirements of these therapies. This cross-disciplinary approach enables a nuanced regulatory strategy, addressing the complexities of novel combination therapies like RAPs and aligning with evolving guidelines. They ensure compliance with evolving guidelines by crafting a cross-disciplinary regulatory strategy that comprehensively addresses safety, efficacy, and quality requirements tailored to the unique challenges of RAPs. The ultimate success of RAPs in clinical practice will rely on overcoming these barriers through concerted efforts, paving the way for a new era of targeted, radiotherapy-

enhanced chemotherapy with improved patient outcomes and minimized side effects.

In conclusion, the integration of RAPs with emerging radiotherapy techniques and precision oncology holds immense promise to transform cancer treatment. RAPs offer a unique opportunity to optimize therapy, enhance patient adherence, and expand radiotherapy's applicability while minimizing systemic toxicity. However, to fully realize their potential, early-phase human clinical trials are essential to validate their safety and efficacy. These trials will be crucial in demonstrating RAPs' ability to improve treatment outcomes and provide a new standard of care. The urgency of clinical validation is clear—RAPs could revolutionize cancer therapy, offering hope for patients with challenging tumors.

CRediT authorship contribution statement

Qiang Wei: Writing – original draft. **Simiao Qiao:** Writing – review & editing. **Qunfeng Fu:** Writing – review & editing. **Zhibo Liu:** Conceptualization, Writing – review & editing.

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.

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.medp.2025.100076.

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