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PUSHING THE FRONTIERS OF RADIOBIOLOGY: A SPECIAL FEATURE IN MEMORY OF SIR OLIVER SCOTT AND PROFESSOR JACK FOWLER: REVIEW ARTICLE

Expanding the therapeutic index of radiation therapy by normal tissue protection

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ABSTRACT

Normal tissue damages induced by radiation therapy remain dose-limiting factors in radiation oncology and this is still true despite recent advances in treatment planning and delivery of image-guided radiation therapy. Additionally, as the number of long-term cancer survivors increases, unacceptable complications emerge and dramatically reduce the patients' quality of life. This means that patients and clinicians expect discovery of new options for the therapeutic management of radiation-induced complications. Over the past four decades, research has enhanced our understanding of the pathophysiological, cellular and molecular processes governing normal tissue toxicity. Those processes are complex and involve the cross-talk between the various cells of a tissue, including fibroblasts, endothelial, immune and epithelial cells as well as soluble paracrine factors including growth factors and proteases. We will review the translatable pharmacological approaches that have been developed to prevent, mitigate, or reverse radiation injuries based upon the targeting of cellular and signalling pathways. We will summarize the different steps of the research strategy, from the definition of initial biological hypotheses to preclinical studies and clinical translation. We will also see how novel research and therapeutic hypotheses emerge along the way as well as briefly highlight innovative approaches based upon novel radiotherapy delivery procedures.

INTRODUCTION

Cancer causes 8.2 million deaths each year globally. Today, most of the anti-cancer therapeutic improvements are achieved by combined treatment modalities, in which radiation therapy remains a cornerstone and is delivered with curative intent in 50% of cancer patients. During the past 20 years, ballistics and imaging improvements have enabled the individualized treatment of patients with a precise and conformal delivery of the dose to the tumor. These technological improvements have greatly enhanced the irradiation therapeutic index. Nevertheless, the level of dose that can be delivered, and accordingly, the possibility of achieving local control over the tumor, is still limited by the toxicity induced to normal (surrounding) tissues. In addition, the combined treatment protocols currently used against cancer are associated with an enhanced risk of toxicity. As the number of cancer survivors increase, preventing and reducing the treatment's side effects is a priority. The present

review provides a list of validated strategies as exhaustive as possible that have been validated to prevent, mitigate, or reverse radiation-induced toxicity in preclinical and clinical studies, together with several new options provided by novel types of radiation therapy.

Cancer causes 8.2 million deaths each year globally. Today, most of the anticancer therapeutic improvements are achieved by combined treatment modalities, in which radiation therapy remains a cornerstone and is delivered with curative intent in 50% of cancer patients. During the past twenty years, ballistics and imaging improvements have enabled the individualised treatment of patients with a precise and conformal delivery of the dose to the tumor. These technological improvements have greatly enhanced the irradiation therapeutic index. Nevertheless, the level of dose that can be delivered, and, accordingly, the possibility of achieving local control over the tumour, is still limited by the toxicity induced to normal (surrounding) tissues.

Figure 1. Biology-driven strategy to identify therapeutic approaches. RNS, reactive nitrogen species; ROS, reactive oxygen species.

- 1- Early inhibition of radiation-induced acute cell death and depletion is presented in part 1.
- 2- Regulation of the redox imbalance is presented in part 2.
- 3- Regulation of inflammatory and fibrogenic signals are presented in part 3.

In addition, the combined treatment protocols currently used against cancer are associated with an enhanced risk of toxicity. As the number of cancer survivors increase, preventing and reducing the treatment's side effects is a priority. The present review provides a list of validated strategies as exhaustive as possible that have been validated to prevent, mitigate, or reverse radiation-induced toxicity in preclinical and clinical studies, together with several new options provided by novel types of radiation therapy.

POST-RADIOTHERAPEUTIC NORMAL TISSUE INJURY

Typical side effects are systemic in the case of drug therapies, whereas radiation-induced normal tissue damages are local or locoregional and can be divided into early and late side-effects. Typically, in the clinic, early effects occur during the time-course of the treatment or within a few weeks of the completion of a fractionated radiotherapy schedule. These effects include skin erythema, dry or moist desquamation of the skin, mucositis, nausea, diarrhea, edema or headaches. Late effects are expressed after latent periods of months to years, and include radiation-induced fibrosis, atrophy, and vascular damage (Figure 1). Likewise, in pre-clinical models, radiation-induced normal tissue injury can be dichotomized into an acute inflammatory phase followed by a late chronic phase characterized by both chronic inflammation and fibrosis. The complications varies from undetectable to highly disabling levels for the patient, inducing a loss of function of the altered organ¹ depending upon extrinsic factors such as variations in the dose delivered, changes in the treatment volume or dose fractionation as well as intrinsic factors such as

individual radiation sensitivity and the presence of comorbidity factors.²⁻⁵

In order to reduce toxicities, it is important to understand the mechanisms underlying the radiation injury of normal tissues. While cell death is the primary and intended effect of ionizing radiation on tumor cells, the deleterious effects of irradiation on normal tissues comprise a cascade of molecular, cellular, and tissue events that spans for a long time after exposure,⁶ and has been compared to a "complex wound".⁷ However, whereas wound repair has three distinct stages that include a clotting/coagulation phase, a restoration phase with fibroblast migration/proliferation, and a final remodeling phase where the normal tissue architecture is restored (Figure 1), this cascade of finely-tuned processes is disrupted post-radiation therapy. Tissue response to irradiation depends upon the intrinsic sensitivity of the various cellular compartments (direct cell death) that compose the organ and the complex crosstalk established in between all these compartments (indirect functional effects). For instance, rapid renewing compartments, like the epithelial layers and bone marrow, show an acute response to irradiation. This is due to a severe depletion of actively dividing upstream progenitor compartments, ultimately resulting in a loss of replenishment of downstream mature cell pools.^{8,9}

In contrast, in connective tissues where cellular turnover is low, radiation injury may be expressed months or even years after exposure if cell death occurs when the cellular division is attempted. The functional consequences are a result of non-lethal effects on different intra- and extracellular molecules and

changes in gene expression in irradiated cells, synthesis of paracrine factors leading, *e.g.* to direct inactivation of anticoagulant molecules, activation of latent growth factors such as transforming growth factor, $TGF\beta_1$, and activation of proteases. The various steps involved in this cascade can constitute valuable therapeutic targets to modify normal tissue injury and enhance the outcome of radiation therapy. In the following sections of this review, we will focus our attention on selected targets, explain their pathophysiological relevance and report the therapeutic strategies that have been used over the past decades. We also discuss how the selection of the timing of administration depends upon the pathway targeted and precludes the selection of the appropriate drug.

Treatment of normal tissue injury- terminology

Before reviewing available treatment options developed to modulate radiation-induced toxicity, it is important to precisely define the possible types of intervention that depend on the time of administration relative to the time of radiation exposure and the appearance of symptoms.⁶ Prophylactic agents or protectors are administered before radiation exposure, mitigators are administered shortly after exposure, but before symptoms arise, while curative treatments are given after the appearance of symptoms as shown in Figure 1.

INHIBITING RADIATION-INDUCED ACUTE CELL DEATH AND DEPLETION

To prevent and inhibit radiation-induced toxicity, the early prevention of epithelial, endothelial, and stem cell deaths is one of the primary options that can be proposed. These interventions imply the administration of drugs delivered prophylactically (Table 1) including inhibitors of pro-apoptotic molecules, such as the transient blockade of p53 (Pifithrin);^{10,11} the inhibition of p-53 upregulated modulator of apoptosis (Puma Inhibitors) or the inhibition of the ceramide pathway in endothelial cells.^{16,17} Alternatively, stimulation of antiapoptotic molecules such as NF- κ B by the flagellin-derivative, CBLB502^{18,19} protects microvascular endothelial cells from radiation-induced death. Similarly, the enhancement of endothelial cell radiation resistance can be achieved by blocking the TSP1/CD47 pathway.²⁰ Other cytoprotective therapies such as keratinocyte growth factor, fibroblast growth factor and glucagon-like peptide-2 treatment^{15,21,22} also have proven efficacy, and prevent the development of normal tissue toxicity. For instance, keratinocyte growth factor administration has been validated and shown to stimulate cell proliferation and promote epithelial cell survival along with the differentiation of oral mucosa, both in pre-clinical and clinical trials.^{23,24} Furthermore, it displayed a beneficial off-target effect by decreasing reactiveoxygen species (ROS) levels and stimulating DNA repair.^{23,24}

These strategies prevent the alteration of an organ's structure and function, and/or enable the rapid restoration of the tissue, markedly when stem cells are protected and activated. Activation of deleterious cascades will be interrupted, avoiding the excessive release of inflammatory mediators and dramatic tissue injury. However, two major drawbacks are associated with these strategies: primarily, there is a high risk of tumor protection and

secondly, the acute rescue of heavily damaged cells could have a delayed detrimental impact on normal tissue structure and function, including the occurrence of a secondary cancers.

RESTORING THE REDOX EQUILIBRIUM IN TISSUE AFTER RADIOTHERAPY

Another important strategy that has been extensively explored to counteract radiation injury involves antioxidant molecules and scavengers aiming at restoring the redox equilibrium of the tissue, immediately after irradiation or at later time points (Table 2).

Direct interactions of ionizing radiation with biological matter induces excitations and ionizations resulting in the ejection of electrons from biomolecules. In addition, indirect interactions occur through ionization of the water and represent the major part of the radiation's effects on the biological matter. Both effects lead to free radical formation. In addition to this rapid burst of free radicals that occurs immediately following radiation, persistent and prolonged increase in reactive oxygen species/reactive nitrogen species (ROS/RNS) is also observed after irradiation. While ROS/RNS in physiological conditions do perform useful functions such as cell proliferation and differentiation^{41,42} and are involved in homeostatis processes such as wound healing,⁴³ when ROS production escalates beyond a certain threshold and becomes persistent, the antioxidant response is not sufficient to reset the system to the original level of redox homeostasis. These high levels of ROS/RNS result in pathological stress to tissues and cells^{2,44} by acting as messenger molecules in cytoplasmic signalling pathways, and by direct effects on transcription.⁴⁵ These elevated concentrations of ROS/RNS not only cause DNA damage, but also alter proteins, lipids, carbohydrates, and complex molecules.

At acute time point post-irradiation, early hypoperfusion occurs due to vascular changes, endothelial cell damages^{16,46} and escalated oxygen consumption, a consequence of increased cellular metabolism. It generates tissue hypoxia, which further exacerbates the injury.⁴⁷ Then, as time passes, the system may still reach an equilibrium associated with higher ROS concentrations called chronic oxidative stress,⁴⁸ which does not really involve a loss of homeostasis but rather a chronic shift in the level of homeostasis. The high level of ROS/RNS are immediate activators of the fibrogenic signals including transcriptional activation of one of the most potent fibrogenic growth factor $TGF\beta_1$,^{49,50} that subsequently upregulates collagen synthesis and perpetuates self-induction and autocrine induction of another potent fibrogenic growth factor, connective tissue growth factor (CTGF).⁵¹ Activation of inflammation mediators has also been described in the case of a high ROS level status, leading to deleterious chronic inflammation in the irradiated tissue. These observations support the fact that radiation-induced late effects are partially propelled by a chronic oxidative stress⁴⁸ induced at late stages by the redox imbalance that occurs in the tissue as a result of intrinsic hypoxia⁵² which further enhance the redox imbalance.

Given the central and persistent role played by the loss of redox equilibrium in the tissue response to irradiation, targeting redox

Table 1. Therapeutic strategies that inhibit acute cell death and depletion

Substance	Intervention/ administration route	Function	Pre-clinical results/ clinical use	References
Pifithrin- α pifithrin- μ	Prophylaxis/protection Oral application of drugs	Inhibition of p53-induced apoptosis	Radiophylaxis/protection in mice after total body irradiation	10,11
KGF-1 (palifermin), KGF-2 (repifermin), FGF-20 (velifermin)	Prophylaxis/protection Oral application of recombinant proteins	Suppression of apoptosis	Reduced mucositis by KGF-1 in patients after whole body irradiation, failure of KGF-2 and FGF-20 in clinical studies	10
Synthetic triterpenoids (bardoxolone methyl (BARD) and 2-cyano-3,12-dioxoooleana-1,9(11)-dien-28-oic acid-ethylamide)	Prophylaxis/protection Oral application	Reduced apoptosis by activation of transcription factor NRF2	Radiophylaxis/protection in mice after total body irradiation	10,12
PUMA inhibitors	Prophylaxis/protection Lentiviral vector	Suppression of apoptosis by inhibition of PUMA	Radiophylaxis/protection <i>in vitro</i> in germ cell tumor line NCCIT; murine hematopoietic progenitor cell line 32D cl 3, and human lymphoblast cell line TK6	10,13
Recombinant MDR1	Prophylaxis/Protection Lentiviral vector	Suppression of apoptosis	Radiophylaxis/protection <i>in vitro</i> in human hematopoietic stem cells	10,14
CBLB502/entolimod	Prophylaxis/protection Oral application	Reduced apoptosis by activation of transcription factor NF- κ B	Radiophylaxis/protection in mice and rhesus macaques; clinical study terminated by financial sponsor	10
GLP-2	Prophylaxis/protection	Trophic factor	RIF gut in rats	15

FGF, fibroblast growth factor; GLP, glucagon-like peptide; KGF, keratinocyte growth factor; MDR, multiple drug resistance; NRF, nuclearre spiratory factor; PUMA, p-53 upregulated modulator of apoptosis; RIF, radiation-induced fibrosis.

Table 2. Therapeutic strategies that prevent, mitigate, and reverse normal tissue injury by modulation of the redox equilibrium

Substance	Intervention/ administration route	Mechanism	Pre-clinical results/clinical use	References
Amifostine	Prophylaxis/protection Oral application of prodrug, mainly activated in normal cells	Scavenger of free radicals	Prophylaxis/protection against xerostomia during radiotherapy of head and neck cancer	25–27
Curcumin, ellagic acid, and bixin	Prophylaxis/protection, mitigation	ROS scavenger	RIF lung in rats and mice	28
Dietary flaxseed	Prophylaxis/protection, mitigation	ROS scavenger	RIF lung in rats and mice	29,30
Glutathione (GSH)	Prophylaxis/protection Oral application of GSH esters or reduced GSH	Scavenger of free radicals (hydroxyl)	Conflicting results in animal models regarding radioprotective effects	10
Genistein	Mitigation	ROS scavenger	RIF lung in rats	31,32
Soy Isoflavone (83.3% genistein, 14.6% daidzein and 0.26% glycitein)	Mitigation	ROS scavenger	RIF lung in mice	33
SOD therapy 1. Recombinant protein 2. Gene therapy 3. SOD mimetics AEOL10150, Eukarion compounds, JP4–039	Mitigation treatment	Detoxification of superoxide	1. Clinical trials recombinant protein not available anymore 2. Radioprophylaxis/protection by viral delivery of SOD2 in preclinical models; validation of plasmid delivery of SOD2 in clinical studies (suspended) 3. RIF lung in rats	5,31,34,35
Pentoxifylline + vitamin E+/ clodronatePentoxifylline +γ-tocotrienol	Treatment	Antioxidants, improves blood flow, anti- inflammatory, TNF-α and TGF-β1 inhibition	Clinical evidence but lack of randomized trialClinical trials starting (e.g. NCT02230800)	36–40

RIF, radiation-induced fibrosis; ROS, reactive oxygen species; SOD, super oxide dismutase; TGF, transforming growth factor; TNF, tumor necrosis factor.

imbalance, ROS/RNS, and hypoxia is an obvious therapeutic option with applications during any of the steps of the cascade as shown in Table 2. Treatment with hyperbaric oxygen (HBO)⁵³ and antioxidant therapy^{54–56} were both successfully used despite their apparent antagonistic mechanism of action. HBO induces transient tissue hyperoxia (typically ~2 h day⁻¹) that should not overcome natural antioxidant defenses,⁵⁷ but may help to remobilize tissue remodelling by activating signaling molecules in transduction cascades (see the review)⁵⁸ and stimulate angiogenesis.⁵⁹ In addition, the results from recent clinical trial show no benefit of HBO on lymphoedema.^{60,61} Antioxidant therapies are based on a different mode of action and aim at scavenging ROS. Initial studies with amifostine^{25–27} and bovine liposomal Cu/Zn superoxide dismutase showed antifibrotic efficacy associated with TGFβ inhibition.⁶² More recent trials investigated the benefits of tocopherol isoforms (Vitamin E analogs) such as high-dose alpha-tocopherol combined with pentoxifylline and clodronate,^{36,37} and γ-tocotrienol (GT3).³⁹ In addition to their antioxidant action, both strategies have displayed off-target benefits with protective endothelial activity^{63,64} and miRNA regulation.⁶⁵ Interestingly, the efficacy of GT3 is enhanced when combined with pentoxifylline.⁶⁶ Lastly, hypoxia-regulating molecules such as 2-methoxyestradiol have been shown to downregulate HIF1α-mediated Smad activation and inhibit radiation-induced lung fibrosis in mice.⁶⁷

TARGETING INFLAMMATORY AND FIBROGENIC SIGNALS INDUCED BY RADIOTHERAPY

The third type of strategy is based upon the understanding of the pathophysiological processes (cellular and molecular) governing normal tissue toxicity. This knowledge has provided us with tools to improve the therapeutic ratio of radiation therapy, and biology-driven efforts have enabled the development of translatable therapeutic approaches to prevent, mitigate, and even reverse radiation injury based upon the targeting of signalling pathways. The relevance of these various signalling pathways to the pathogenesis and maintenance of radiation injury has been extensively and recently reviewed in several articles.^{40,51,68,69} Therefore, we will focus on recent results that highlight the relevance of immune cells in response to irradiation. Elucidating the impact of radiotherapy on the immune compartment and subsequent immunomodulation is nowadays one of the most promising strategies for improving anticancer treatment,⁷⁰ and recent studies suggest that it may also enhance the differential effect of radiotherapy.

The importance of myeloid cells in the radiation-induced response has been proposed and the role of macrophage reprogramming by radiotherapy has been demonstrated.^{71–73} Macrophage phenotypes are highly dependent upon the

microenvironment and recent publications have revealed their complexity.⁷⁴ In fibrotic tissue, macrophages do display immunosuppressive properties, secrete large amounts of the fibrogenic mediator TGF- β 1⁷⁵ that activates the Smad pathway, and stimulate downstream fibrogenic genes such as CTGF and PAI-1.⁷⁶ The macrophages isolated from bronchoalveolar fluid from patients undergoing thoracic irradiation spontaneously released platelet-derived growth factor, another important fibrogenic growth factor.⁷⁷ Several older studies have suggested possible benefits of macrophage depletion using clodronate liposomes.⁷⁸ The reduction of the number of macrophages by clodronate in wounded tissue indeed reduced excessive scar formation and delayed cutaneous wound healing.⁷⁹ Fromm and colleagues⁸⁰ showed that the oral administration of clodronate (bisphosphonate) significantly reduced bone marrow fibrosis, and in the early 2000's Delanian and Lefaix successfully administered clodronate in combination with a pentoxifylline-vitamin E treatment, and showed improved efficacy in the treatment of radiation-induced fibronecrosis.^{81,82}

More recent data validate and refine this strategy bringing molecular highlights and a biological rationale for macrophage targeting in the management of radiation-induced normal tissue complications.^{83,84} Recently, the P Huber group showed

that blocking CTGF with a specific antibody (FG-3019) was able to attenuate radiation-induced pulmonary remodeling and reverse fibrosis. Interestingly, they showed that this treatment was associated with the abrogation of M2-like macrophages influx.⁸³ We extended these findings and recently characterized the contribution of pulmonary macrophages to radiation-induced pulmonary fibrosis.⁸⁴ We showed that the populations of pulmonary macrophages are heterogeneous and their contribution to fibrosis is complex. A differential phenotype for alveolar and interstitial macrophages was indeed shown along with a specific fibrogenic contribution of interstitial macrophages but not alveolar macrophages. Ultimately, selective targeting of interstitial macrophages with CSF1R mAb was shown to display antifibrotic action.

Lastly, an overview of the drugs that have been used to prevent and mitigate radiation damages as well as the drugs that have been successfully used to reverse radiation-induced complications, fibrosis in particular, are provided in [Tables 3](#) and [4](#). The impressive list of compounds shows the vitality of the research in this field with an impressive rate of translational/clinical studies ([Table 4](#)) using curative strategies. Three parameters have probably fostered this progress: first, the irreversibility of fibrosis was challenged by many cellular and experimental

Table 3. Therapeutic strategies that prevent and mitigate normal tissue injury by modulation of inflammatory and fibrogenic signals

Substance	Intervention/administration route	Mechanisms	Pre-clinical results/clinical use	References
Ambroxol	Prophylaxis/protection	TNF- α and TGF- β 1 inhibition	Clinical trial	85
Taurine	Prophylaxis/protection	TGF- β 1 and collagen inhibition	RIF lung in mice	86,87
IL-11 (targeted administration)	Prophylaxis/protection	TGF- β 1 and collagen inhibition	RIF gut in mice	88
Hirudine	Prophylaxis/protection	Thrombin inhibition	RIF gut in rats	89
Halofuginone	Prophylaxis/protection	TGF- β 1 inhibition	RIF skin in mice	90
Octreotide	Prophylaxis/protection	Somatostatin analogue	RIF gut in rats	91
Soluble TGF- β type II receptor	Prophylaxis/protection	TGF- β 1 inhibition	RIF gut in mice	92
ACE inhibitors angiotensin II blockers	Prophylaxis/protection and mitigation	Angiotensin II modulator and inhibition of TGF- β	Clinical trial	93
Methylprednisolone, dexamethasone, ibuprofen)	Prophylaxis/protection and mitigation	Anti-inflammatory	RIF kidney and heart in rats and rabbits	94-96
Gefinitinib	Mitigation	EGFR inhibition-TKi	RIF lung in rats enhances inflammation but decreases fibrosis	97
LY2109761	Mitigation	TGF- β R1 inhibition-S/TKi	RIF lung in mice Reduces inflammation and fibrosis	98
Chitosan/DsiRNA targeting TNF- α	Mitigation	TNF- α inhibition	RIF subcutaneous in mice	99

ACE, angiotensin converting enzyme; EGFR, epidermal growth factor; RIF, radiation-induced fibrosis; TGF, transforming growth factor; TKi, tyrosine kinase inhibitor; TNF, tumor necrosis factor.

Table 4. Therapeutic strategies that reverse normal tissue injury by modulation of inflammatory and fibrogenic signals

Substance	Intervention/administration route	Mechanisms	Pre-clinical results/clinical use	References
All -trans-retinoic acid	Prophylaxis/Protection and treatment	TGF- β 1, IL-6 and collagen inhibition	RIF lung and gut in mice	100,101
Angelica sinensis	Prophylaxis/Protection and treatment	TGF- β 1 inhibition	RIF lung in mice	102
Antibody against CTGF	Prophylaxis/protection and treatment	CTGF inhibition and macrophages depletion	RIF lung in mice	83
CSFR1 inhibition	Treatment	Macrophages depletion	RIF lung in mice	84
Interferon gamma (low dose)	Treatment	Collagen production inhibition	Small Clinical trial	103
Pirfenidone	Treatment	TGF- β 1, PDGF, b-FGF, EGF, TNF- α inhibition	Clinical trial open	104
Heparine and Wwarfarine	Treatment	Anticoagulant	Small clinical trial open	105
Colchicine	Treatment	Collagen production inhibition	Clinical trial	106
Statins	Treatment	Vascular protector, anti-inflammatory, TGF- β 1 inhibition	Clinical evidence but lack of randomized trial	93,107
Pentoxifylline + vitamin E+/- clodronate Pentoxifylline + γ -tocotrienol	Treatment	Antioxidants, improves blood flow, anti-inflammatory, TNF- α and TGF- β 1 inhibition Macrophages depletion	Clinical evidence but no randomized trial Clinical trials starting(e.g., NCT02230800)	36-40

CSF, colony stimulating factor; CTGF, connective tissue growth factor; EGF, epidermal growth factor; FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; RIF, radiation-induced fibrosis; TGF, transforming growth factor; TNF, tumor necrosis factor.

studies; second, curative strategies are clinically interesting as they do not interfere with anticancer treatments through possible tumor protection; third, they can be delivered to the targeted population of patients that need it.

USE OF NOVEL RT APPROACHES TO PROTECT NORMAL TISSUES

Aside from novel biological interventions, improvements in physics have been critical for protecting normal tissue, enhancing differential effects, and making progress on tumor control. Novel technologies based upon sophisticated instrumentation used in conjunction with imaging, such as stereotactic body radiotherapy, have helped to protect normal tissue by reducing the irradiated volume and more accurately targeting the tumor. More complex and expensive technologies, such as proton and carbon ion therapies, take advantage of their specific pattern of energy deposition in the biological matter and are already implemented in clinical practice. Other novel ideas and approaches have also been recently proposed such as dose rate escalation.^{[108,109](#)} This last approach should be translatable into clinical application soon, which illustrates the broad range of opportunities that exist in radiation therapy, and highlight the need for interdisciplinary working teams composed of biologists, physicists, and physicians.

IGRT and Stereotactic body radiotherapy

Image-guided radiation therapy (IGRT) has been a major advancement in radiotherapy and makes it possible to visualize

a target volume (tumor + margins) and the surrounding organs at risk before treatment and during the treatment course. Modifications in the targeted volume as well as movements are taken into consideration, and can be compensated for and even halted in the case of percussion-assisted RT (PART) recently described by Péguret et al.^{[110](#)} This innovative technique induces a long-lasting apnea-like suppression of respiratory motion for up to 10–11 min without inducing any physiological side effect.^{[111](#)} The pilot clinical study reported an interesting advantage of percussion-assisted RT compared to free-breathing or maximal-inspiration techniques coupled with three-dimensional conformal RT, SBRT or VMAT irradiations, in breast cancer, lung cancer, and lung metastases patients. IGRT combined with motion management is associated with the prescription of highly conformal doses (intensity modulated radiotherapy, IMRT) which are meant to drastically decrease the irradiated volume and the dose delivered to the normal tissue. Different techniques may be used to follow the anatomical structures, with or without the addition of fiducial markers, starting from fluoroscopy, to CT, MRI, PET-CT, ultrasounds or optical tracking.

Studies have shown an improvement in toxicity prevention on several tumor sites^{[112](#)} and especially in prostate cancer patients,^{[113](#)} even if these results can sometimes be controversial.^{[114](#)} It is difficult, however, to differentiate the IGRT from the IMRT effects, and several studies have exposed this limitation^{[115,116](#)} through their explanation of the reduced occurrence of xerostomia due to PTV margin reductions.

Proton therapy

Ballistic features of protons prevent normal tissue toxicities

Due to the protons' in-depth dose deposition curves, one can expect to prevent normal tissue toxicity and therefore, decrease normal tissue injury. By now, more than 100,000 patients have been treated worldwide with proton beam therapy (PBT) in approximately 20 centers. This means that evaluating PBT superiority in terms of normal tissue protection is now feasible.¹¹⁷

Proton therapy has been largely used to treat ocular tumors such as uveal carcinoma, making it an alternative to enucleation or ocular brachytherapy thus sparing visual acuity.^{118–121} Concerning skull base tumor treatments, different Swiss and American studies have shown that the percentage of patients treated with PBT and suffering from temporal lobe injury is reduced compared to conventional X-Ray radiation therapy and IMRT.^{122–124}

In pediatrics, the large volume of tissue exposed to low-doses induces severe and non-acceptable long-term injuries such as neurocognitive impairments, hearing loss, hormonal dysfunctions, infertility and secondary malignancies. Therefore, PBT seems to be ideal for treating pediatric patients. Dosimetry reconstruction comparing X-Rays, IMRT and PBT treatment plans show an improved dose distribution¹²⁵ with PBT which, according to modeling, could be responsible for at least a 2-fold, and up to a 15-fold reduction in secondary malignancies because of normal tissue irradiation.¹²⁶ Interestingly, a Chinese study estimated that IMRT is the technique that displays the highest risk of secondary malignancy (30%), while PBT was linked to only a 4% risk of developing a radio-induced cancer.¹²⁷

PBT has also shown encouraging results concerning the neurocognitive toxicity of whole and partial brain irradiation in children. The reduction of the irradiated normal brain volume enables a drastic reduction of the deleterious effects in the early cognitive outcome 1 year post-RT compared to photon therapy. These results were observed for IQ, verbal comprehension, and working memory.¹²⁸

Lung cancer treatments with PBT show a possibility of increasing dose and subsequent anti-tumor efficacy with reduced adverse effects—especially dermatitis, esophagitis, and pneumonitis—compared to classical X-ray treatments. For breast cancer treatments, PBT plans decrease by 71–81% and 75–99% of lung and heart irradiation respectively. Moreover, dose to the contralateral breast and dose to the whole body were also drastically reduced.^{129–134} All of these results suggest a decrease in radio-induced lung dysfunctions and cardiopathies, but also for the occurrence of secondary malignancies, showing that proton therapy, by decreasing dose to the normal tissue, reduces the occurrence of radiation-induced injury, and can improve the therapeutic ratio of radiation therapy.

Carbon ion therapy

Increase the relative biological effectiveness and the therapeutic ratio

The efficacy of high linear energy transfer (LET) particles in radiation therapy has been investigated since the late 70s.¹³⁵ The

first clinical trial was conducted in Japan in 1994,¹³⁶ and since then, more than 13,000 patients have been treated with carbon ion radiation therapy.¹³⁷ Carbon ions display the same pattern of dose deposition as protons, with a Bragg peak deposition of the dose, low entry and exit doses as well as a fall-off after the Bragg peak that is significantly steeper compared to protons. The dose deposited beyond the Bragg peak is higher compared to protons because of the nuclear interaction and fragmentation. One other additional interesting property of Carbon ions is their high LET, which is directly linked to their RBE that ranges between 2.0 and 3.5. The high RBE is certainly beneficial for tumor control, but can be detrimental to the normal tissue and therefore increase complications. Still, exploring the differential effects of Carbon ions is of great interest, and pioneering work performed more than 20 years ago by the Denekamp team has shown that reducing the number of fractions lowers the RBE for both normal tissue but not for the tumor.¹³⁸ Hypofractionation in carbon ion radiation therapy would increase the therapeutic ratio, and provides a strong biological rationale for Carbon ion use.^{138–142}

FLASH-RT

Opportunities to improve the efficacy of radiation therapy via the development of new irradiation techniques may have been under explored. Today, modern radiation therapy devices still use the same technology of electron acceleration in waveguides as half a century ago. However, the recent development of proton therapy facilities and the use of high LET ions exemplify some of the possibilities that are currently opened. Previous experiments conducted with short pulses of X-rays on lymphocytes,¹⁴³ or more recently, conducted with protons on human-hamster hybrid cells and skin cells,^{144–146} including microchannel radiotherapy that operates at 200 Gy.s⁻¹ dose-rate,¹⁴⁷ showed fewer cytogenetic damages and significantly protected normal tissue from radiation-induced acute and long-term damages.

In line with these experiments and with the objective of fostering innovation in radiation therapy, we have been the first to propose a completely novel modality of irradiation, named FLASH radiotherapy. It markedly increases the differential effect between tumors and normal tissues, and is able to destroy tumors with the same efficiency while providing better protection to the normal tissues and preventing side effects. Indeed, using several pre-clinical models,^{108,109} we have shown that an ultrahigh dose rate delivery of irradiation was able to protect normal tissues in mice (lung, brain, gut and skin), in pigs (skin), and in a clinical trial performed in cats bearing spontaneous cancers for whom a major protection of normal tissues was observed, while maintaining a strong anti-tumor efficacy¹⁰⁸ (Vozenin et al, under revision; Montay-Gruel et al. in prep). This effect has been called the Flash effect. The Flash effect has been confirmed by another team from Stanford University (USA)¹⁴⁸ and we found similar observations reported more than 40 years ago.^{149,150} In addition to this radiobiological advantage, the ultrashort duration of dose deposition overcomes the potential problems associated with tumor motion, and can then enhance RT delivery accuracy. Currently, only a few devices are able to deliver an ultrahigh dose rate irradiation across a large volume of tissue; experimental electron Linacs of 4/6 MeV have limited therapeutic applications

due to their energy profile and limited in-depth dose deposition (Kinetron, Orsay, Fr, PMB-Alcen, Pegnier, France; Oriatron, Lausanne, CH, PMB-Alcen, Pegnier, France; modified clinical accelerator, Stanford, USA).^{109,151} However, several technological improvements are ongoing to upgrade those systems and develop clinical transfer.^{152,153}

CONCLUSION

The selective protection of normal tissue function using modern targeted radiotherapy is a powerful approach to improve cure rates and simultaneously enhance the quality of life of long-term cancer survivors. Nowadays, high precision radiation therapy induces a drop in the rate of complications. In parallel, advancements in radiobiology have deciphered the complexity of the biological response induced by tissue exposure to ionizing radiation, and enabled the identification of therapeutic targets. These processes include profound microenvironment remodeling with alteration of the vascularization, perfusion/hypoxia, inflammation, modulation of immune compartments and stromal remodeling. Therefore, currently, well-selected combination strategies that target distinct pathogenic pathways induced by irradiation at specific time points of the pathogenic process can be proposed,

and the next challenge will be to develop rational radiotherapy–drug combinations to maximize the therapeutic impact. The management of RT complication has also reached the era of personalized medicine and in many centers in Europe (France, UK, CH for instance), patients presenting with complications are managed in the frame of multidisciplinary consultations to adapt the best therapeutic answer for each specific situation. Simultaneously, novel radiotherapy approaches such as the ultra-high dose rate, Flash RT, have been developed, offering the potential to radically change the way radiation therapy is employed and delivered over the next few years.

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