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Review

Small Bowel Dose Constraints in Radiation Therapy—Where Omics-Driven Biomarkers and Bioinformatics Can Take Us in the Future

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Abstract: Radiation-induced gastrointestinal (GI) dose constraints are still a matter of concern with the ongoing evolution of patient outcomes and treatment-related toxicity in the era of image-guided intensity-modulated radiation therapy (IMRT), stereotactic ablative radiotherapy (SABR), and novel systemic agents. Small bowel (SB) dose constraints in pelvic radiotherapy (RT) are a critical aspect of treatment planning, and prospective data to support them are scarce. Previous and current guidelines are based on retrospective data and experts' opinions. Patient-related factors, including genetic, biological, and clinical features and systemic management, modulate toxicity. Omic and microbiome alterations between patients receiving RT to the SB may aid in the identification of patients at risk and real-time identification of acute and late toxicity. Actionable biomarkers may represent a pragmatic approach to translating findings into personalized treatment with biologically optimized dose escalation, given the mitigation of the understood risk. Biomarkers grounded in the genome, transcriptome, proteome, and microbiome should undergo analysis in trials that employ, R.T. Bioinformatic templates will be needed to help advance data collection, aggregation, and analysis, and eventually, decision making with respect to dose constraints in the modern RT era.

Keywords: gastrointestinal toxicity; radiation therapy; omic alterations; gut microbiome; biomarkers

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1. Introduction

Pelvic radiation therapy (RT) plays an important role in the treatment of urological, gynecological, and colorectal malignancies. RT improves survival outcomes and reduces the risk of disease recurrence [1–3], but it is associated with radiation-induced acute and chronic dose-limiting lower gastrointestinal (GI) toxicity, including diarrhea, abdominal cramping, fibrosis, obstruction, ulceration, bleeding, and fistula formation [4,5]. Several factors have impacted the landscape of small bowel (SB) dose constraints, including paralleling technological and systemic therapy advances in oncology; intensity-modulated radiation therapy (IMRT); stereotactic ablative radiotherapy (SBRT); and increasingly varied novel personalized systemic management. With the progression of advanced image-guided RT technology, efforts to minimize radiation doses to organs at risk (OARs) have resulted in smaller PTV margins; however, bowel constraints have remained mostly unaltered. Given that practice and decision-making are evidence-based, and the evidence has not kept pace with advancement in this space, dose constraints have become an area of controversy. We aim to review the pathophysiology and dose response of small bowel injury in the current landscape of SB constraints, and look into the future, where robust proteomic, metabolomic, and microbiome surrogates for acute and late bowel toxicity may be explored and eventually implemented into protocols and the standard of care.

2. The Pathophysiology and Dose Response of Small Bowel Injury

Radiation-induced toxicity is mainly explained by clonogenic cell death and apoptosis in crypt cells, resulting in the insufficient replacement of the villus epithelium, the breakdown of the mucosal barrier, leading to mucositis, and the prominent inhibition of compensatory proliferative reactions [6,7]. The pathophysiology of small bowel injury is illustrated in Figure 1. Acute radiation-induced toxicity corresponds to mucosal injury, where the normal villous epithelium of the intestine is renewed by non-functional cells, which leads to the loss of the barrier effect, and consequently, to abdominal pain and accelerated intestinal transit. Conversely, late radiotoxic effects include a combination of submucosal fibrosis and vascular degeneration [8]. A correlation between the RT dose to the small bowel and acute GI toxicity rates has been previously demonstrated [4,9]. Intensity-modulated radiation therapy (IMRT) has been found to be associated with a lower dose to the small bowel [10–12] (Figure 1). This translated into better clinical outcomes measured based on grade 2 and $\geq$3 acute GI toxicity [13,14]. Therefore, utilizing IMRT in pelvic RT may lower GI toxicity rates and potentially allow for safe dose escalation, and it is widely accepted as a relatively safe and effective RT method. However, RT-induced toxicity is still a matter of concern. In an RCT by Sauer et al.; acute grade 3 to 4 GI toxicity rates were 12% and 18% among patients receiving preoperative and postoperative RT for rectal cancer, respectively [15]. Concurrent chemotherapy further adds to RT-induced SB toxicity, as demonstrated in several studies. For example, in a Gynecologic Oncology Group study, cervical cancer patients who received 45 Gy pelvic RT alone experienced a 5% rate of Grade 3 to 4 GI toxicity vs. 14% observed in those who received RT plus weekly cisplatin (40 mg/m^2) [16]. Data that quantitatively outline the specific additive impact of chemotherapy on RT in terms of acute and late toxicity remains poorly defined. Although the small bowel represents the most important dose-limiting structure in pelvic, R.T.; prospective data on the actual dose constraints to be employed with IMRT are scarce, and ideal dose–volume constraints to minimize GI toxicity have not been well quantified. The data in this area are outdated and largely retrospective, limiting the ability of clinicians to leverage them in developing modern treatments. Attempts have been made to define clinical aspects that are predictive of RT toxicity. Several prior studies have evaluated predictors of GI morbidity during, R.T.; including lower body mass index (BMI) in gynecologic cancer [17]. Jadon et al. [18] performed a systematic review of dose–volume predictors and constraints for late bowel toxicity following pelvic radiotherapy. In their review, many studies found no correlation with OAR dose parameters and late bowel toxicity at all, and they concluded that clear recommendations for these organs cannot be made, due to lack of correlation between studies. They stated that rather than dose–volume predictors, other considerations such as the inherent radiosensitivity of individual patients may be the main predictors of toxicity; these, however, are not currently being measured, and thus, data will continue to lag behind evolving technology in this area.

An example of this is the use of stereotactic ablative radiotherapy (SABR), which has expanded over the past decade. Although phase III data are still awaited, phase II trials and pooled analyses demonstrate good clinical outcomes in terms of progression-free survival and overall survival in different indications [19,20]. SABR enables good avoidance of OARs and is considered a relatively safe treatment; however, severe toxicity has also been reported [21,22]. Small bowel dose constraints present a critical issue in pelvic SABR. The HYPO-RT-PC trial showed that a dose of 42.7 Gy to the prostate delivered over 2.5 weeks (6.1 Gy per fraction) caused grade 2 and worse GI toxicity rates that ranged from 1 to 16% [23].

The High Dose per Fraction, Hypofractionated Treatment Effects in the Clinic (HyTEC) group has established select OAR constraints for different OARs, not including the SB [24]. Other commonly used guidelines include the American Association of Physicists in Medicine Task Group (AAPM-TG) [25], Timmerman constraints [26,27], and the United Kingdom (UK) Consensus Guidelines [28] (Figure 2). Gerhard et al. identified substantial variability for certain OARs in a systematic review of organ-at-risk dose constraints in SABR in ongo-

ing clinical trials [29]. Data are actively evolving, and it is likely that in the coming years, as trial data mature, more clear guidelines may emerge.

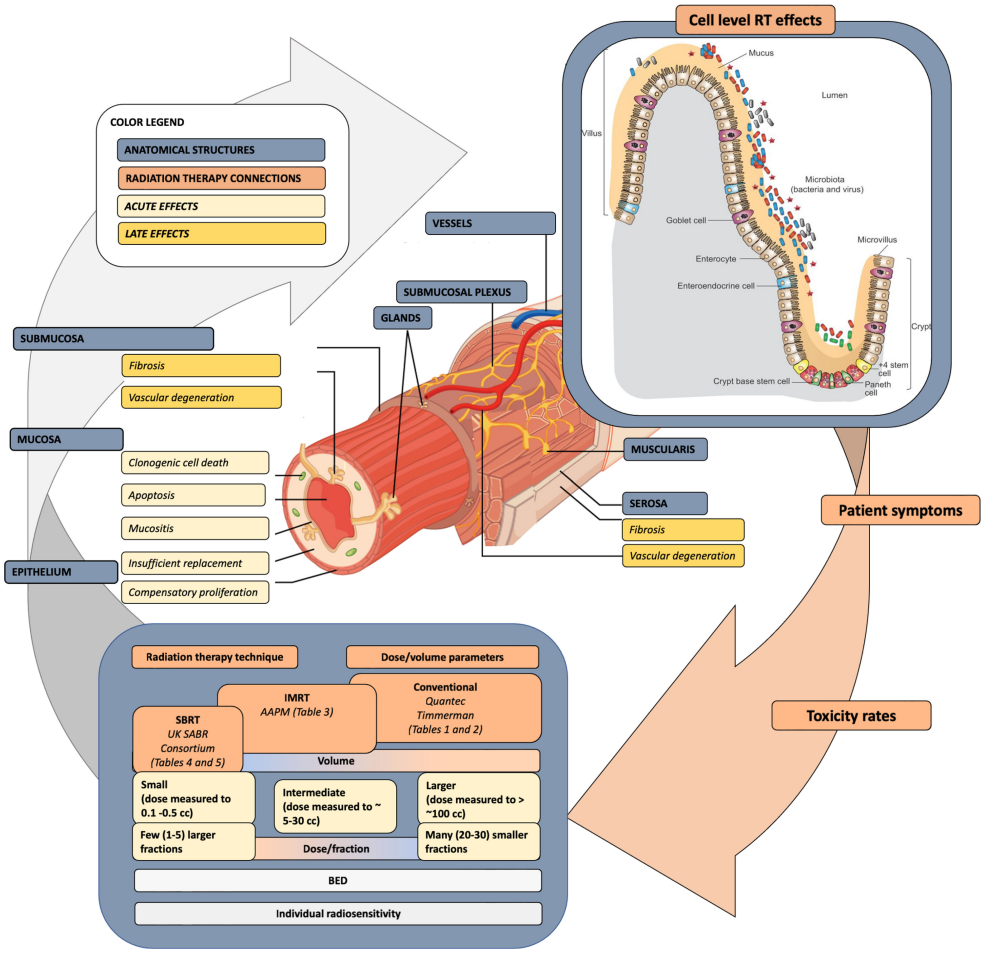

Figure 1. The pathophysiology of small bowel injury [30–32].

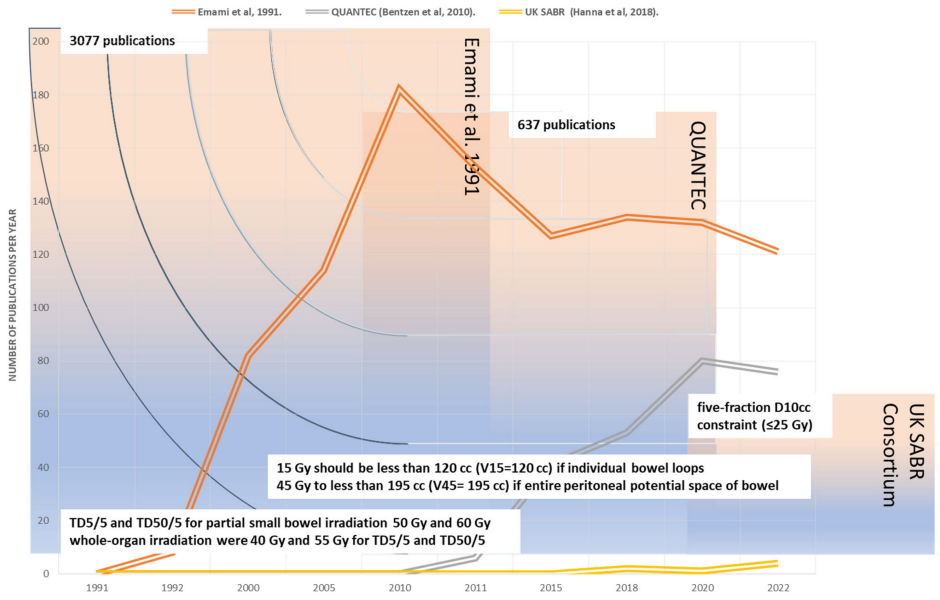

Figure 2. Current small bowel constraints—a walk back in time [28,33,34].

3. Current SB Constraints—A Walk Back in Time

To be able to direct future data generation and analysis to optimize constraints, it is worth examining the evolution and ongoing use of constraints. SB constraints have evolved over time (Figure 2). The oldest dose constraints date back to 1991, when Emami et al. [33] published the tolerance doses for the irradiation of 1/3, 2/3, or the entirety of various organs, mainly based on consensus of clinical experience or opinions, as high-quality clinical data were scarce. They estimated doses with a 5% or 50% risk at 5 years (TD5/5 and TD50/5, respectively) for late SB toxicity. The TD5/5 and TD50/5 for partial SB irradiation were 50 Gy and 60 Gy, respectively, whereas the constraints for whole-organ irradiation were 40 Gy and 55 Gy for TD5/5 and TD50/5, respectively. These constraints, which no longer reflect the currently available imaging and treatment planning availability and utilization, still make up the bulk of published data on SB constraints (Figure 2), with many, mainly retrospective, clinical studies published on dose–volume–outcome analysis. The Emami constraints still far outweigh any other constraint construct, although there is an observable transition to the Quantitative Analysis of Normal Tissue Effects in the Clinic (QUANTEC).

In 2010, the QUANTEC Steering Committee was formed to provide a critical overview of the current state of knowledge and to produce practical guidelines for clinicians' decision making based on dose–volume parameters. This effort representing an updated iteration of constraints was meant to address the availability of 3D planning. QUANTEC guidelines advise that the absolute volume of SB receiving 15 Gy should be less than 120 cc (V15 = 120 cc) if individual bowel loops are outlined, or 45 Gy to less than 195 cc (V45= 195 cc) if the entire peritoneal potential space of the bowel is outlined [34].

The Quantitative Analysis of Normal Tissue Effects in the Clinic (QUANTEC) review is most widely quoted today, but given the number of publications in comparison with Emami, it safe to conclude that data generated and available for analysis that carry QUANTEC as compared to Emami annotation are relatively few (Figure 2). A 2010 review summarized the available dose–volume data for bowel toxicity [34], including six papers which examined the dose–volume relationship of bowel with acute bowel toxicity in the treatment of rectal or gynecologic cancer [9,35–39]. All studies were retrospective, except Huang et al.; which was a non-randomized prospective trial [38]. It derived recommendations based on the available data at that time and provided the foundation for normal tissue toxicity prediction.

QUANTEC recommendations are still quoted today, however, in the era of image-guided IMRT, and SBRT extrapolation to individual clinical scenarios is increasingly difficult. This is complicated by understanding that irradiated SB volume, as well as various patient-related factors, including genetic, biological, and clinical features, may modify the clinical response and predict toxicity. In addition, additive effects of novel systemic and immune system-mediating agents are poorly understood.

SABR represents the extreme end of small-volume, high-dose, personalized, image-guided therapy (Figure 1). It is far from the older conventional but relatively homogenous dose distribution that dose constraints are based on. In SABR, evidence-based OAR dose constraints have not been published. In 2008, Timmerman was the guest editor of a *Seminars in Radiation Oncology* issue on the topic of hypofractionation and published a table of constraints that are mostly engineered, titled "Mostly Unvalidated Normal Tissue Constraints for SBRT" [26]. These guidelines were widely adopted worldwide and have been updated in many publications [27,40] (Tables 1 and 2). Task Group 101 of the American Association of Physicists in Medicine (AAPM) has also published a summary of suggested dose constraints for various critical organs to outline treatment guidelines for SBRT [25] (Table 3). A UK SABR Consortium consensus for normal tissue constraints was published in 2017 [28], mainly derived from AAPM Task group 101 [25]. A review and update of the previous consensus was then published in 2022 [41], and included constraints and related works made available since the previous publication (Tables 4 and 5). Several minor changes have been made for SB constraints, including the following: The five-fraction D10 cc constraint ($\leq$25 Gy) has been moved to become optimal, because this dose (EQD2

40 Gy, based on α/β = 3 Gy) has been delivered safely to much larger volumes of small bowels in clinical practice, without excessive short- or long-term toxicity [42,43]. In addition, the previous optimal five-fraction D5 cc constraint (also EQD2 40 Gy) has been removed. Normal tissue dose constraints in IMRT and SABR are still evolving, and only limited data exist to support evidence-based guidelines and recommendations in scenarios currently faced in the clinic by radiation oncologists.

Table 1. Timmerman's dose constraints of the duodenum **.

Number of Fractions	Volume (cm^3)	Volume Max (Gy)	Max Point Dose (Gy) *	Endpoint (Grade $\geq$ 3)
1	<5	17.4	22	Ulceration
2	<5	20	26	
3	<5	22.5	30	
4	<5	25	33.2	
5	<5	26.5	35	
8	<5	31.2	42	
10	<5	33.9	45	
15	<5	39	51	
20	<5	42	54	
30	<5	45	60	

* "Point" is defined as $\leq$ 0.035 cm^3. ** One third of the "native" total organ volume (before any resection or volume-reducing disease, whichever is greater).

Table 2. Timmerman's dose constraints of the jejunum/ileum **.

Number of Fractions	Volume (cm^3)	Volume Max (Gy)	Max Point Dose (Gy) *	Endpoint (Grade $\geq$ 3)
1	<30	17.6	20	Enteritis/obstruction
2	<30	19.2	24	
3	<30	20.7	28.5	
4	<30	22.4	31.6	
5	<30	24	34.5	
8	<30	28.8	40	
10	<120	33.9	41	
15	<120	39	46.5	
20	<120	42	50	
30	<120	45	54	

* "Point" is defined as $\leq$0.035 cm^3. ** One third of the "native" total organ volume (before any resection or volume-reducing disease, whichever is greater).

Table 3. AAPM dose constraints of the, S.B.

	Max Critical Volume above Threshold	One Fraction		Three Fractions		Five Fractions	
		Threshold Dose (Gy)	Max Point Dose (Gy)	Threshold Dose (Gy)	Max Point Dose (Gy)	Threshold Dose (Gy)	Max Point Dose (Gy)
Duodenum	<5 cc	11.2	12.4	16.5 (5.5 Gy/fx)	22.2 (7.4 Gy/fx)	18 (3.6 Gy/fx)	32 (6.4 Gy/fx)
	<10 cc	9		11.4 (3.8 Gy/fx)		12.5 (2.5 Gy/fx)	-
Jejunum/Ileum	<5 cc	11.9	15.4	17.7 (5.9 Gy/fx)	25.2 (8.4 Gy/fx)	19.5 (3.9 Gy/fx)	35 (7 Gy/fx)

Table 4. UK SABR Consortium consensus for duodenum constraints.

	2017			2022		
	Constraint	**Optimal**	**Mandatory**	**Constraint**	**Optimal**	**Mandatory**
1 fx	-	-	-	D0.1 cc	-	12.4 Gy
	-	-	-	D10 cc	-	9 Gy
3 fx	DMax (0.5 cm^3)	-	<22.2 Gy	D0.1 cc	-	22.2 Gy
	D5 cm^3	-	<16.5 Gy	D10 cc	-	11.4 Gy
	D10 cm^3	-	<11.4 Gy			
5 fx	DMax (0.5 cm^3)	-	<35 Gy	D0.1 cc	33 Gy	35 Gy
	D1 cm^3	<33 Gy	-	D10 cc	25 Gy	-
	D5 cm^3	<25 Gy	-			
	D9 cm^3	<15 Gy	-			
	D10 cm^3	-	<25 Gy			

Table 5. UK SABR Consortium consensus for SB constraints.

	2017			2022		
	Constraint	**Optimal**	**Mandatory**	**Constraint**	**Optimal**	**Mandatory**
1 fx	-	-	-	D0.1 cc	-	15.4 Gy
				D5 cc	-	11.9 Gy
3 fx	DMax (0.5 cm^3)	-	<25.2 Gy	D0.1 cc	-	25.2 Gy
	D5 cm^3	-	<17.7 Gy	D5 cc	-	17.7 Gy
	D10 cm^3	-	<11.4 Gy			
5 fx	DMax (0.5 cm^3)	<30 Gy	<35 Gy	D0.1 cc	30 Gy	35 Gy
	D1 cm^3	-	-	D10 cc	25 Gy	-
	D5 cm^3	<25 Gy	-			
	D9 cm^3	-	-			
	D10 cm^3	<25 Gy	-			

4. The Biomarker and Bioinformatics Frontier

While biomarkers for tumor diagnosis, response, and progression are often the subject of investigation, biomarkers of radiation response in normal tissue receive less attention. In the context of bowel radiation dose response, leveraging omics approaches is challenging. One challenge is the diversity of presentation by tumor type, location, and radiation dose. The other is the variability in relevant biomarkers that may be leveraged in different types of specimens, including blood, serum, urine, stool, and tissue (Figure 3).

Specimens are also affected by significant confounders, including the patient's diet, comorbidities, and systemic management. Metabolic differences that have been described between patients with gastrointestinal malignancies and normal controls have been connected to tumor location and stage [44]. Therefore, measurable omic alterations between patients harboring malignancy and receiving RT to the gut may be harnessed to identify patients at risk for greater acute and late toxicity. This may translate to the possibility of personalized treatment and perhaps the option of biologically optimized dose escalation, given the mitigation of the understood risk. The gut microbiome has recently been found to present links to RT response and the development of gastrointestinal mucositis, possibly via immunomodulation [45]. More recent work in mice showed that following high-dose radiation, "elite-survivors" among the mice harbored distinct gut microbiota that devel-

oped after radiation and protected against radiation-induced damage and death, in both germ-free and conventionally housed recipients [46]. In a recent clinical review discussing gastrointestinal toxicities following pelvic radiotherapy, the toxicity of RT was related to dysbiosis of the gut microbiome [47]. Thus, it is conceivable that the gut microbiome can uniquely allow for both the diagnosis and prediction of acute and late toxicity, as well as their modulation, providing a unique opportunity to identify actionable biomarkers of normal tissue change in response to, R.T. A template for data acquisition and analysis in this space that may help guide the identification of small bowel and radiation response biomarkers is data obtained and analyzed in the context of inflammatory bowel disease [48]. Studied here were serum (anti-Saccharomyces cerevisiae antibodies (ASCAs) and anti-neutrophil cytoplasmic antibodies (ANCAs)) and fecal biomarkers (calprotectin and lactoferrin), all associated with intestinal inflammation. Biomarkers in this clinical context can and should be analyzed at the genome, transcriptome, and proteome levels. The proteome is particularly appealing, since proteins drive processes involved in radiation response and toxicity, and their alteration over time may be employed to study acute and late effects. Large-scale proteomic analysis in the context of the small bowel as normal tissue has, to date, not been published. Potentially significant proteomic signals that have previously been published include proteomic changes that occur during intestinal cell maturation along the crypt–villus axis (villin, ezrin 40, CRBPII 29, and FABP1 29) [49], microbiome and metabolic alteration (pyrimidine metabolism and tryptophan compounds with a gut microbiome metabolic contribution) [50], and fibrostenotic and inflammatory phenotypes [51]. Although proteomic panels examining total-body and partial-body exposure to radiation have been analyzed [52], a large-scale data analysis of radiation-induced alteration of the gut proteome in a histological, radiation dose-dependent, or anatomical location-dependent manner has, to date, not been published. The acquisition of biospecimens from patients receiving RT to the gut can advance data gathering and the analysis of acute and late toxicity, and may address ongoing unmet needs for data collection that reflects clinical and RT parameters while keeping pace with the rapid evolution of dose and fractionation and the heterogeneity that permeates both clinical and planning data.

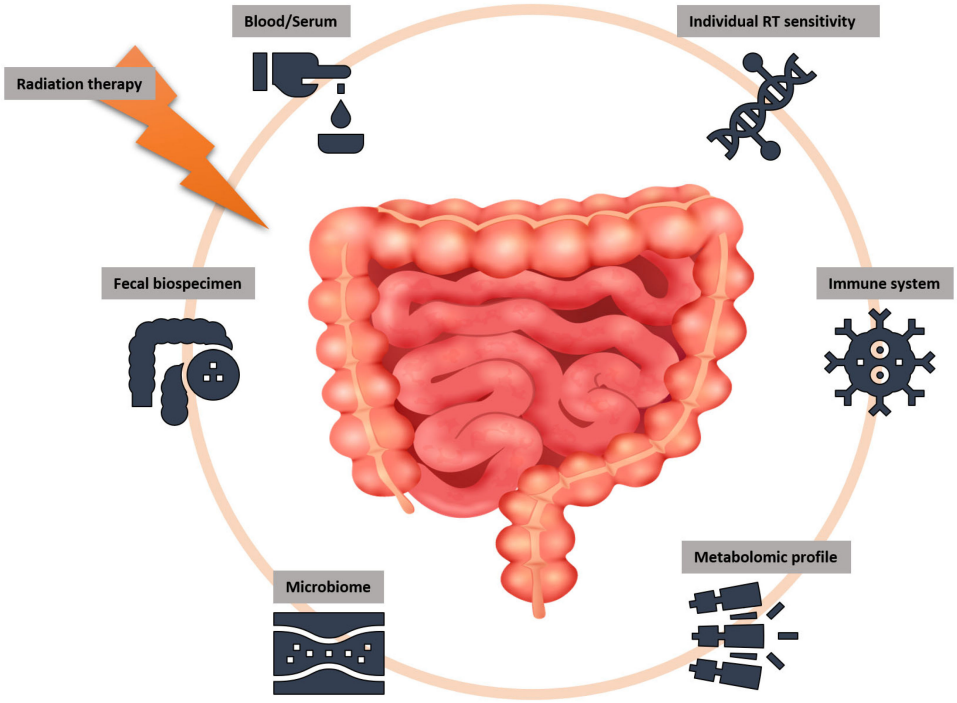

Figure 3. The biomarker and bioinformatics frontier—toward biomarkers of radiation response and predictors of acute and late toxicity.

Genomic analysis is increasingly being employed with the increased availability of next-generation sequencing (NGS), and we have an increased understanding of how genomic variants impact small-bowel cancer [53]. Considering the potential overlap between conditions that increase the risk of bowel cancer, such as inflammatory bowel disease (including Crohn's disease), where radiation therapy is usually discouraged or used cautiously, it has become important to collect genomic data. These data can then be compared to identify the key factors contributing to bowel toxicity. Specifically, it would be valuable to classify the inflammatory component in various groups, including individuals without any medical conditions, those with inflammatory bowel disease, those with cancer, and those who eventually receive radiation therapy [54].

To date, the transcriptomic profile of the bowel in response to radiation therapy (RT) has not been thoroughly studied. Nevertheless, RNA sequencing is becoming increasingly popular due to its cost-effectiveness. This technology has the potential to enable the analysis of how different radiation doses affect the bowel's response, including the impact of the radiated volume. It may even allow for a detailed examination at the single-cell level. Such an approach would significantly enhance our knowledge of both normal tissue reactions and how tumors respond to radiation therapy [55]. Large-scale proteomic profiles are growing in several tumor sites [56–60]. Analyses of the microbiome are connected on three fronts, the gut, the tumor, and normal tissue, as reviewed by Liang et al. [61] in the context of glioma. The interactions between microbiomes at each of these levels are not well understood. In comparison, linking alterations in the gut microbiome to small bowel (SB) toxicity may be facilitated in malignancies where radiation therapy (RT) is directed at the gut, thanks to the ability to measure the RT dose to the bowel using RT contouring segmentation data from the RT treatment planning system in various clinical contexts. A recent study (REIMAGINE) characterized the small bowel microbiome in a segmental manner compared to the stool microbiome using luminal aspirates and stool samples [61], finding that the small bowel microbiome was distinct from that of stool and also varied by bowel segment. Analyses like this and the generation of microbiome models can significantly advance our understanding of normal small bowel function at the omic and microbiome levels if adequately aggregated with bioinformatic approaches with RT dosimetry data. As discussed briefly in earlier sections, it is imperative to emphasize that substantial differences exist in the microbiome composition between patients diagnosed with cancer and those who remain cancer-free, both in cancers that inhabit the gut and those originating elsewhere [62]. In their study, Zhao and colleagues conducted a comparative analysis involving six distinct colorectal cancer (CRC) cohorts, encompassing a total of 353 patients, each paired with corresponding normal mucosal samples. Their analysis revealed the presence of varying numbers of bacterial genera, ranging from 205 to 562 depending on the dataset. Subsequently, they discerned two distinct patient–microbe interaction patterns, referred to as P0 and P1, which exhibited differences in microbial alpha and beta diversity. Furthermore, the researchers delved into microbial correlation networks and examined their relationships with clinical factors such as age, gender, and BMI. In a separate study, Iadsee et al. [63] compared the microbiota profiles of 80 patients, 25 with CRC, 33 with adenomatous polyps, and 22 healthy controls, characterizing mucosal tissue and stool samples. The authors identified Erysipelatoclostridium ramosum (ER) as a stool-based biomarker using qPCR to predict CRC, with a specificity of 72.7% and a sensitivity of 64.7%. These analyses, such as those outlined within the context of the microbiome, generate extensive datasets that hold significant potential for applications in areas like treatment response. These datasets can also be valuable in clinical scenarios involving radiation therapy (RT) targeting the small bowel. However, it is important to note that these data sources—the microbiome, bowel segments, the RT dose to bowel segments, and clinical data—are currently separate and unconnected.

To harness their full potential, bioinformatic approaches capable of integrating these disparate data types need to be developed. Such integration can substantially enrich the depth of information available and, in the long term, facilitate the establishment of RT

dose constraints based on biomarkers. For instance, there are currently approximately 30 ongoing research studies dedicated to exploring various aspects of microbiome analysis in conjunction with radiation therapy (RT), as detailed in Table 6 [64]. Among these studies, only 2–3 are directly focused on RT applications to the pelvic region, like prostate, colorectal, and renal cell carcinoma, and four studies address non-pelvic primary sites such as breast, head and neck, and glioblastoma. It is noteworthy that all studies within this domain involve the collection of stool samples, while the head and neck study also collects serum samples. The interventions in these studies typically align with standard-of-care management, although one study explores the administration of checkpoint inhibitors or dietary supplements.

Table 6. Currently recruiting trials aimed at the microbiome and RT [64].

Study Name	Intervention	Microbiome Analysis
Pelvic Primaries		
Study to Detect Changes in Urinary and Gut Microbiome During Androgen Deprivation Therapy and Radiation Therapy in Patients with Prostate Cancer	Androgen deprivation therapy and Radiation Therapy	Stool and urine
L. Plantarum 299v and Gut Microbiome, Diarrhea, and Clostridioides Difficile Infection in Colorectal Cancer Patients	Dietary Supplement: Sanprobi IBS®/chemotherapy and radiation	Stool
The Gut Microbiome and Immune Checkpoint Inhibitor Therapy in Solid Tumors (NSCLC, MM, TNBC or RCC, Stage 1–4)	Checkpoint inhibitor, immune	Stool
Non-Pelvic primaries		
The Association Between Radiation Dermatitis and Skin Microbiome in Breast Cancer Patients	Post-operative radiotherapy	Skin
Assessing the Impact of the Microbiome on Breast Cancer Radiotherapy Toxicity	Stool sample and skin swab sample	Skin and stool
Correlation of Fecal Microbiome and Its Metabolites with Outcome of Radiotherapy in Head and Neck Carcinoma	Radiotherapy	Stool and serum
THERApeutic Outcomes Related to Gut microBIOME in Glioblastoma (GBM) Patients Receiving Chemo-radiation (THERABIOME-GBM)	Chemoradiation	Stool

However, it is important to highlight that the evolving studies in this field generally consist of relatively small patient cohorts, often comprising fewer than 100 patients. An exception to this is the solid tumor study, which encompasses a variety of cancer types (non-small-cell lung cancer (NSCLC), multiple myeloma (MM), renal cell cancer (RCC), and triple-negative breast cancer (TNBC)) at any stage and aims to enroll a substantial cohort of 800 cancer patients. It is worth noting that the administration of RT in this particular study is not a specifically studied intervention.

To fully harness the potential of these diverse datasets, it is imperative to implement bioinformatics frameworks capable of elucidating connections between RT dosage and various factors, including the microbiome, proteome, metabolome, and their dynamic alterations. These should be considered alongside clinical variables and specific interventions, including, R.T. While several omic data types, such as proteomic and transcriptomic data, benefit from established databases like IPA, STRING, and GSEA for data analysis, RT dose information for specific anatomical structures is seldom available for correlative analysis.

One notable example of such integration can be found in a study on lung cancer [65], where the researchers integrated microbiome, metabolome, and proteome analyses in non-small-cell lung cancer, ultimately identifying the, P. copri-nervonic acid/all-trans-

retinoic acid axis as a contributing factor in the pathogenesis of NSCLC. In this study, the DNA analysis of fecal microorganisms employed 16S rRNA gene sequencing, with annotations accomplished using the Ribosomal Database Project classifier. The functions of the intestinal flora were identified using PICRUST software. In parallel, serum and tissue metabolomics analyses were conducted, and various databases were utilized for proteomic data classification, while yet another database was employed for identifying metabolic pathways.

Similarly, in an integrated analysis of the fecal metagenome and serum metabolome in colorectal cancer and adenoma [66], Chen et al. identified reprogramming patterns associated with the serum metabolome in patients with CRC. It is worth noting that neither of these analyses specifically delved into RT dosage, but they both vividly exemplify the unprecedented capabilities of bioinformatics in identifying data patterns intricately linked to malignancy development. Consequently, investigating alterations in such patterns following RT administration emerges as a pivotal endeavor, promising to enhance dose constraints and potentially refine our ability to safely escalate radiation doses.

The integration of omics-driven biomarkers and bioinformatics in radiation therapy planning holds great promise for personalized cancer treatment. However, several limitations and challenges must be addressed to harness the full potential of these approaches. First, the variability in data generation platforms, experimental techniques, and sample sources can lead to inconsistencies and inaccuracies. Second, the lack of standardized protocols for data collection, processing, and analysis across different laboratories and institutions can impede the comparability of results. Third, developing universally accepted algorithms and frameworks for data interpretation remains an ongoing challenge, as the complexity of the biological information requires advanced algorithms and computational models. The implementation of omics-driven biomarkers into routine radiation therapy planning requires validation in large, diverse patient cohorts, overcoming regulatory hurdles, demonstrating clinical utility, and ensuring cost-effectiveness. Table 7 demonstrates the available data aimed at omics-driven biomarkers and, R.T.

Table 7. Available data aimed at omics-driven biomarkers and, R.T.

Study/Author (Year)	Study Design	Biomarker	Outcome
Dublineau [67] (2004)	Pre-clinical	Gastrointestinal peptide plasma levels	Changes in gastrin and neurotensin plasma levels were associated with structural alterations in the stomach and ileum, respectively.
Onal [68] (2011)	Prospective	Plasma citrulline levels	Citrulline concentration changes significantly differed during treatment according to RTOG intestinal toxicity grades.
West [54] (2011)	Review	Genetic variation (SNPs)	It is impossible to say with certainty whether any genetic variations predispose patients to toxicity.
Guo [46] (2020)	Pre-clinical	Gut microbiome	Gut microbiome contributes substantially to radioprotection.
Liu [45] (2021)	Review	Gut microbiome	Underlying mechanisms are still obscure, and more research is needed to clarify the links between the gut microbiome and variations in RT response.
Oh [47] (2021)	Review	Gut microbiome	Toxicity of RT was related to dysbiosis of the gut microbiome.
Sproull [52] (2022)	Analysis	Plasma proteomic expression profiles	Identified novel panels of radiation-responsive proteins useful for predicting radiation exposure.

5. Conclusions

The analysis and identification of potential predictors for acute and late lower gastrointestinal (GI) toxicity holds immense significance. This endeavor is essential to establishing robust dose parameters that can dynamically adapt to minimize patient toxicity as our repertoire of treatment strategies evolves and becomes increasingly personalized. To pave the way for the development and validation of such parameters, it is imperative to gain a profound understanding of the interplay between potential omic surrogates and clinical responses, fostering optimization of the therapeutic ratio and the management of treatment-related side effects.

We strongly advocate for the comprehensive analysis of radiation-induced alterations in the gut proteome, microbiome, and other omic data types. These measurable omic changes among patients receiving radiation to the small bowel (SB) could help identify individuals at greater risk of experiencing acute and late toxicity. It is paramount that biospecimens for omic and microbiome analyses are prospectively collected as a standard practice across all oncologic studies. This proactive approach enables future analyses and offers the potential for an enhanced biological understanding. It is particularly crucial because both dose escalation and technological advancements are advancing rapidly, often outpacing our ability to comprehensively aggregate and understand data from treatment planning systems.

The establishment of a well-thought-out strategy for acquiring omic and microbiome data is paramount to continuing the safe escalation of radiation doses and the personalization of treatments while simultaneously improving the management of side effects. In this modern era of radiation therapy, a unified bioinformatic toolkit that encompasses both omic and microbiome data is indispensable. Such a toolkit would empower decision-makers to make more informed choices, resulting in a biologically optimized therapeutic ratio. This integrated approach is vital to supporting better decision making with a biologically optimized therapeutic ratio in the modern RT era.

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Abbreviations

AAPM-TG	American Association of Physicists in Medicine Task Group
BED	Biologically effective dose
BMI	Body mass index
CRC	Colorectal cancer
EQD2	Equivalent dose at 2 Gy per fraction
GI	Gastrointestinal
HyTEC	Hypofractionated Treatment Effects in Clinic
IMRT	Image-guided intensity-modulated radiation therapy
IPA	Ingenuity pathway analysis
MM	Multiple myeloma
NGS	Next-generation sequencing
NSCLC	Non-small-cell lung cancer
OARs	Organs at risk
PTV	Planning target volume
QUANTEC	Quantitative Analysis of Normal Tissue Effects in the Clinic
RCC	Renal cell carcinoma

RCT	Randomized controlled trial
RT	Radiation therapy
SABR	Stereotactic ablative radiotherapy
SB	Small bowel
TNBC	Triple negative breast cancer
TD5/5	Tolerance dose resulting in 5% risk of severe complications within 5 years after irradiation
TD50/5	Tolerance dose resulting in 50% risk of severe complications within 5 years after irradiation
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins
GSEA	Gene Set Enrichment Analysis

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