Future of Team-based Basic and Translational Science in Radiation Oncology

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Future of Team-based Basic and Translational Science in Radiation Oncology

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To further optimize radiotherapy, a more personalized treatment towards individual patient's risk profiles, dissecting both patient-specific tumor and normal tissue response to multimodality treatments is needed. Novel developments in radiobiology, using in vitro patient-specific complex tissue resembling 3D models and multiomics approaches at a spatial single-cell level, may provide unprecedented insight into the radiation responses of tumors and normal tissue. Here, we describe the necessary team effort, including all disciplines in radiation oncology, to integrate such data into clinical prediction models and link the relatively "big data" from the clinical practice, allowing accurate patient stratification for personalized treatment approaches.
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Introduction

The low-hanging fruits of radiation oncology have been discovered already, leaving more difficult challenges to discover novel paradigms. More accurate tumor irradiation with various irradiation qualities, dose tempo, improved target volume, and margin definition, (re)planning techniques, NTCP modeling, understanding of drug-radiation interaction, and precision medicine have been explored and implemented. These have improved tumor control and prolonged survival for many tumor sites[1-3] but have consequently resulted in an increased number of cancer survivors dealing with late normal tissue side effects. To further optimize cancer treatment, radiotherapy must be more personalized toward the individual patient's risk profile, dissecting both patient-specific tumor and normal tissue radiosensitivity, immune response, and drug/immune therapy radiation interaction. This to ensure improved tumor response with less normal tissue effects for cancer patients receiving radiation. Moreover, predicting treatment response and advancing knowledge on post-treatment tumor and normal tissue micro-environment reactions is essential to optimally facilitate tumor control while stimulating normal tissue regeneration.[4-6] Unprecedented, combined expertise is required to achieve this in its full capacity. In other words, radiation biologists, molecular biologists, stem-cell biologists, geneticists, computational biologists, bioinformaticians, translational biologists/physicians, physicists, oncologists, radiologists, pathologists, surgeons, and radiation oncologists need to work together to delve into complex problems. This perspective paper focuses on novel developments in personalized radiotherapy from a radiobiologist's view matched with the clinical translational counterpart, where clinical information—available from routine clinical practice—can be extracted and used for toxicity or tumor outcome prediction.

Novel technological developments in radio-biology research have yielded unprecedented possibilities to study radiation effects on more complex animal and patient-derived models, ranging from *in vitro* cell lines and organoids to *ex vivo* tissue slices and *in vivo* humanized animal models. This combination of models allows for a better understanding of the damage mechanism and response and a more rapid translation to the clinic due to the closer resemblance of the patients. Next to that, current developments in multiomics approaches allow for an integrative analysis of radiation response parameters, providing unprecedented insight into the radiation responses of tumors and normal tissue. A multiomics approach may combine tumor and normal tissue data sets of genome, proteome, transcriptome, epigenome, metabolome, and/or microbiome to study responses of cells and tissues to, e.g., cancer treatments in a concerted way.

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The possibility of performing radiation research using multiomics at a single cell level in a spatial environment[7,8] will yield novel approaches to optimize radiation responses. The resulting complex biological big data set can be analyzed to find novel associations between biological processes, and indicate biomarkers, providing more in-depth knowledge of radiation response processes. A close interaction between bioinformaticians and computational biologists is needed to analyze these data. Regretfully, for analysis of patient-specific responses, these methodologies are presently too time-consuming and costly for individual prediction.

While generic response predictors/biomarker changes have already been tested in the clinic, this is where the gap with clinical translational research regarding prediction modeling needs to be bridged, as rapid advances in biotechnological and artificial intelligence (AI) methodologies allow the integration of multiomics data to predict treatment responses from diverse dimension levels. Particularly, since the bulk of translational research on the image "biomarkers"—otherwise referred to as radiomics features—that are extracted from routinely acquired imaging[9-11] often convey a similar message as that from the (radio)biological community, e.g., high tumor heterogeneity detected on CT is related to poorer outcomes[12,13]. Additionally, capturing the metabolic activity, FDG-PET imaging features have also shown that higher metabolic values in the tumor are linked to poorer outcome.[14-18] Higher PET intensity was linked to better normal gland tissue outcome, while for lung tissue, higher metabolic activity increases the risk of radiation-induced toxicity.[18-23] This shows how biological processes can be captured in imaging, which is also exemplified by the PET imaging studies aiming to capture hypoxia culture in tumor tissue[24] or Diffusion-Weighted Imaging, which estimates the diffusion of water molecules in tissue to detect tumor tissue or quantify damage.[25-28] Yet, other image biomarkers such as spatial tumor features—e.g., tumor volume, surface, sphericity, and compactness—often highly associated with the tumor response,[11,29] cannot be captured with *in vivo* biological biomarkers, which are typically obtained through biopsies or incisions. Hence, biological, omics, and image biomarkers can be highly complementary to each other in predicting tumor and normal tissue response. Moreover, relating multiomics biomarkers to predictive patient-specific imaging biomarkers extracted from PET-CT imaging data can strengthen individualized outcome prediction, as both suffer from institute and material susceptibility.[30-33] Not only could the current clinical prediction models be enriched with the right biological markers, but the link between the relatively "big data" from the clinical practice could also be delved to identify relevant biological markers that allow accurate patient stratification for personalized treatment approaches (see Fig. 1).

This perspective will address the possibilities available and how (patient-specific) radiobiology and clinical parameters could be combined to yield a rapid and highly individualized prediction of future patient responses.

Biological Approaches

Cell lines have provided invaluable information on cellular radiation response characteristics, DNA damage response, cell cycle, radiosensitivity, etc., and led to one of the most used prediction models, the LQ model.[2] Cell lines will also, in the future, provide us with more mechanistic insight into the molecular response to radiation and combined therapies and can be used to validate big data-derived correlative mechanisms. However, although it is valuable to test molecular mechanisms, they will not provide individual patient-specific response parameters. Moreover, cell lines have performed poorly in establishing normal tissue responses, mainly because no good cell lines represent normal tissue complexity. Fibroblasts have been used to predict normal tissue responses, but besides showing some relation with fibrosis, they have largely failed to describe and predict the complex response of normal tissues. Recently, the landscape has changed with the development of 3-dimensional (3D) culture systems, allowing cell lines to grow as spheroids, showing more realistic cell-cell interactions and the radiation dose response.[34,35] However, even these still consist of only a single cell type, which does not represent the diversity of cell types in tumors or normal tissues. Other models are now available for these.

Organoids are 3D models derived from stem cells capable of self-renewal and contain multiple cell types of a complex structure that resembles the normal or tumor tissue of origin. They recapitulate the characteristics and functionality of the tissue, including genetic mutations.[36] Therefore, organoids represent an excellent model for studying patient-specific tumor and normal tissue radiation responses.[35,37] Organoids can be cultured from embryonic stem cells, induced pluripotent stem cells, animal tissues, and patient biopsies, allowing diagnostics, disease prognosis, treatment outcome prediction, and organoid cell-based therapies.[38] Indeed, organoids can provide treatment prediction for a variety of cancers such as colorectal,[39] head and neck cancer,[40] and pancreatic cancer,[41] while trials on lung cancer, liver cancer, kidney cancer, brain cancer, esophageal cancer, and breast cancer are ongoing.[38]

Normal tissue organoids have also revealed exciting characteristics, as shown with salivary gland and thyroid gland-derived organoids showing low-dose hypersensitivity.[42] Moreover, the feasibility of a patient-tailored evaluation of gastrointestinal response to radiation using human intestinal organoids using comparative transcriptome profiling to photon, proton, and carbon irradiation was shown.[43] Another interesting approach was recently published, where bulk and single-cell RNAseq reveal different transcriptomic changes of organoid-derived cells after photon and proton irradiation.[44] Interestingly, upon proton irradiation, a higher expression INF-β for many tumor sites related genes, cytoplasmic dsRNA, and expression of latent endogenous retroviruses was observed, leading to a robust immune activation and inflammatory response, like what is seen after a viral infection.[45,46] A loss of histone H3K9 methylation and consequential derepression of transposable elements

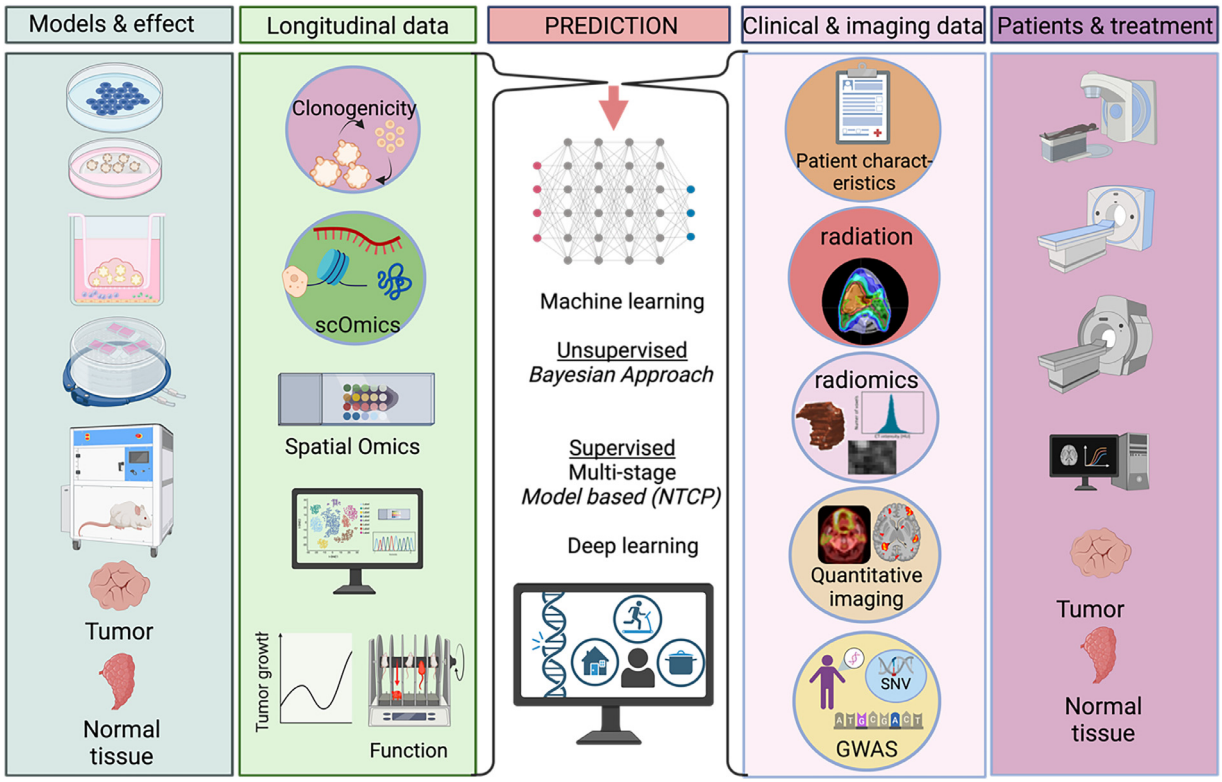

Figure 1 Schematic representation of the potential methods and "bio" markers to predict the patient's treatment response. From the left to the middle, models for radiobiology (blue) allow the collection of data on radiation responses, (cancer) stem cell function, specific cellular and spatial responses, and tumor plus normal tissue responses (green). From the right to the middle in purple, patient treatment planning and dose distribution can be correlated to the patient's characteristic genetic information (pink), imaging data, normal tissue function, and tumor response measurements. The white column in the middle shows how the biology data can be merged with clinical data to establish response prediction models.

accompanied this. This process could be directly related to the stem cell response within the organoids, inducing an enhanced regenerative potential after proton compared to photon irradiation.[44] Interestingly, it was shown that a similar resurrection of endogenous retroviruses was observed during aging, which reinforced cellular senescence.[47] These studies indicate the potential of using organoids to study biological processes involved in the radiation response, allow the detection of biomarkers, and predict patient responses. However, organoids do not represent the complete tissue, as they lack blood vessels, immune cells, and (tumor-associated) fibroblasts. For this, co-culture "assembloids" have been developed to aggregate cells of different lineages able to self-organize, giving rise to more complex multilineage interactive organoids.[48,49] Next to this, a co-culture system where semi-permeable membranes separate organoids from cell types of other lineages may provide additional information.[50] Subsequent postirradiation multiomics will provide novel information on the radiation response of the different cell types and how they interact. Although promising still, these models do not entirely reflect the tissue in its normal state.

Human precision-cut tissue slices represent an organ mini-model that closely resembles the organ from which it is prepared, with all cell types in an original tissue-matrix configuration. Tissue slices have been used for drug discovery and development.[51] They may yield information on metabolism and radiation-drug interactions in normal and tumor tissue. The multicellularity, intact cell-matrix interaction, and metabolism can be used to study the viability and functionality of radiation-affected (e.g., fibrotic) tissue. In capable hands, slices can remain viable for many days.[51] As such, an ex vivo tissue slice culture model of clear cell renal cell carcinoma was developed to elucidate the impact of nivolumab on tumor-infiltrating immune cells,[52] indicating the robustness of the model in identifying patient response to specific therapeutics.

That acts as a decoy hijacking the DNA damage response. It was observed that the drug protected the normal tissue by triggering a G1/S arrest.[53] Regretfully, precision cut slides have a limited viability of about 2 weeks maximum under optimal conditions and may also be overgrown by a specific rapidly proliferating cell type. However, this model may represent a midway between organoids and humanized animal models.

Humanized mouse models are immunodeficient mice co-engrafted with human tumors and innate immune components. They can be used to study patient-specific interactions between engrafted matched tumors and immune cells. They are used in immuno-oncology research with potential for clinical translation.[54] Regretfully, there is a lack of preclinical studies using this model for radiobiology-based research.

However, it could be used to study radiation responses involving the innate immune system, such as the "abscopal effect".[55] Interestingly, using a lung squamous cell carcinoma humanized mouse model, it was shown that targeting NK cells may be an essential strategy for combining radiotherapy with immune checkpoint inhibitors.[56]

However, humanized mouse models show a restricted development of mature innate immune cells and lack HLAs and antigen-specific antibody responses.[54] Next-generation novel humanized mouse platforms may accelerate the research on the immune response to radiation in combination with (new) immunotherapies.

Clinical Approaches

Clinical prediction models are based on clinical tabular data and can predict various treatment outcomes. Examples are clinical models that predict overall survival for head and neck cancer,[57-60] Non-Small Cell Lung Cancer,[61] and oligometastatic disease metastatic disease[62] or biochemical outcome in prostate cancer patients.[63] These models typically incorporate a range of predictors, including patient characteristics (e.g., age, sex, performance score, pack years), tumor characteristics (e.g., AJCC stage, tumor location, Gleason score), and occasionally treatment information (e.g., chemotherapy, radiation dose) to predict a patient-specific risk score. Some also consider semantic imaging features (e.g., extranodal extension) or biomarkers (e.g., HPV status or PSA levels). In contrast to the biological approaches, larger quantities of data are relatively simple to collect, particularly with the institution of efficient standardized follow-up programs and with the (near) future potential of mining all clinical data from electronic health records with natural language processing (NLP) techniques into model-ready tabular data.[64] Big clinical datasets have allowed for robustly trained and validated models supported by large, representative, multi-institutional datasets.[58,60]

Clinical models are often presented in a nomogram format,[57,59,62,65] while graphical user interface applications[66] or web-based applications[58,60] are more sophisticated methods to present the treatment outcome probability.[58,60,66] Regardless, prediction models are rarely used in the clinic but rather indirectly incorporated in our oncologic decision protocols, typically using binary cutoffs of risk factors, e.g., adding concomitant chemotherapy based on the AJCC stage and an age limit only. Yet, mortality and tumor outcome remain highly variable within these defined groups, highlighting the need for improved personalization of treatment with model-based risk estimations.[58]

Normal Tissue Complication Probability (NTCP) models predict radiation-induced side effects that are generally based on pretreatment dose parameters of organs-at-risk, combined with clinical variables, such as age or baseline complaints.[67] Examples are NTCP models that predict xerostomia with mean salivary gland doses[68] with mean lung dose[69] and rectal toxicity with doses related to the substructures within and around the anorectum.[70] Several countries have implemented NTCP models in clinical decision-making to select patients for advanced normal tissue sparing radiotherapy techniques, such as proton therapy.[71] Moreover, the paradigm shift of radiation dose optimization based on NTCP models (model-based optimization) instead of radiation dose objectives may become a commercially available application in the future.[72-74]

Radiomics models are based on features (i.e., image "biomarkers") that quantify patient-specific tissue characteristics from clinical images, translating intensity, texture, and geometric properties region-of-interest into single variables that can be fed into a machine learning modeling pipeline.[9] Radiomics hypothesizes that these high-dimensional features are minable and can quantitatively reveal more information, as "medical images are more than pictures, they are data."[9,75-77] The first radiomics study showed that pretreatment CT-based radiomics features that predict overall survival for nonsmall cell lung cancer patients performed well in an independent lung and a head and neck cancer patient cohort.[10] Several subsequent studies have shown that tumor radiomics features can contribute to the prediction of overall, disease-free, progression-free survival in multiple disease sites: e.g., head and neck,[10,78-81] lung[10,11] cervical,[82] prostate and rectal[83] cancer patients. Gross modo, these studies suggest that high texture values in the tumor, capturing higher tumor heterogeneity, are typically associated with a higher risk of recurrences and poorer survival.[75,81] Geometric features typically indicate that larger and more irregularly shaped (e.g., volume surface, bounding box) tumors are related to recurrences and worse survival rates.[81] Moreover, radiomic features have also been associated with pathology or molecular markers (e.g., HPV)[84–87] and when combined with genomic markers, they can improve prediction.[88] This demonstrates the potential of associating radiomics with biology and highlights the complementary effects it can have in improving the prediction of treatment outcomes.

Radiomics has also been applied for normal tissue response or toxicity, addressing the challenge that patients who receive similar radiation doses to organs can have highly variable toxicity presentations. Radiomics features of the parotid glands obtained from pretreatment CT and ^{18}FDG-PET images were added to the conventional dose-based xerostomia prediction model,[20,89] something that was independently validated.[19,21] This work demonstrated that high heterogeneous CT intensities and low metabolic activity in parotid gland tissue were associated with a higher probability of developing permanent late xerostomia, suggesting that larger ratios of fat-to-functional parotid gland tissue were related to a higher risk of xerostomia. This was later confirmed by the strong relation between fat-related MR-features and late xerostomia.[90,91] Another example, radiomics features that indicated that a large volume of high PET intensity was associated with the development of radiation pneumonitis, suggested that pretreatment subclinical inflammation may already be a risk factor for pneumonitis.[22] In addition, images during treatment have been shown to give useful delta radiomics features to predict toxicities.[92-95]

Clinical implementation of radiomics models has yet to be realized in radiotherapy. One of the major challenges that these models face is imaging and image processing variation.[30,96-98] While the image biomarker standardization initiative (IBSI) has produced guidelines to harmonize image processing, feature definition, and extraction,[33] the variability in imaging acquisition still challenges the robustness of radiomics features[98,99] and likely attributes to why radiomics features fail to predict treatment outcome or validate in some centers.[100-104] Advancements in MRI, such as quantitative modalities like Diffusion Weighted Imaging (DWI) and Dynamic Contrast Enhanced (DCE) MR imaging, can provide meaningful intensity values related to diffusion and perfusion of tissue, respectively.[105-107] These methods are, however, more susceptible to motion and geometric distortion and are limited by noise and resolution.[108] This field needs to mature to propel the field of personalized radiotherapy forward.

Radiomics combined with clinical variable models have improved prediction performance. The radiomics feature can add information to clinical variables such as age, TNM stage, and performance score but can also replace them. The latter was shown by Zhai et al., where the feature major axis length of all lymph nodes (i.e., the maximum length between pathological lymph nodes) better predicted survival than the N-stage.[81] This indicates the potential for quantification of clinical variables and the importance of being wary of multicollinearity (i.e., high inter-associations between the independent variables).[109]

Deep learning models or convolutional neural networks differentiate themselves from conventional machine learning approaches by extracting weights and features directly from the input data, such as 3D imaging and radiotherapy dose data, without predefining variables. Typically, the initial layers of these networks are convolutional layers, applying various imaging filters in sequence. The processed data is then flattened and passed through fully connected layers that perform a function analogous to variable selection.[110,111] Deep learning applications can be found throughout the radiotherapy workflow, from image acquisition segmentation to treatment planning and delivery.[112] Deep learning in radiotherapy outcome prediction is also growing rapidly, with studies focusing on using imaging data to predict tumor responses and overall survival.[110,111,113-116] Moreover, combining imaging and 3D dose distributions as deep learning inputs has been explored for tumor outcome and toxicity prediction.[117-120] Additionally, adding genomics data as e.g., obtained from genome-wide association study (GWAS) can identify genetic differences between patients related to certain toxicities, as has been shown for radiation-induced xerostomia.[121] Adding these may improve prediction. However, deep learning is intrinsically data-hungry, and the required enormous datasets are not readily available in radiotherapy. Even though algorithmic measures (e.g., dropout, permutations) have been implemented to enhance generalizability, this still illustrates the need to train models on collective multi-institutional datasets.

By directly learning from images, deep learning comes with the trade-off of being a "black box" compared to conventional machine learning approaches, as defined features can be more easily related to semantic concepts, such as tumor size and heterogeneity. Deep learning attention maps can provide insights into the focal areas of the deep learning network models, visually highlighting key regions on the input image.[122,123] This can aid the model's reliability by identifying potential biases in predictions and generating hypotheses by showing new regions associated with tumor outcome or toxicity development. The potential to link imaging and outcome variation to genome data or organoid processes is even more exciting. However, explainable AI is still in an early stage, and interpretation can be ambiguous.

Merged Approaches

If we want to move towards a more personalized treatment strategy, we will need to adapt to model-based probability as a marker for treatment selection. It is essential to take personalized radiotherapy to the next level and to alter the radiation dose to the patient's risk, i.e., dose escalation for patients at high risk of treatment failure versus dose de-escalation for low-risk patients. For this, combining clinically derived data and knowledge of biological processes will increase the predictive potential of such models.

Although biology models can be used to describe mechanisms, show potential biomarkers, and predict the response of patients to cancer therapies, regretfully, for patient-specific response prediction most of the biology models may take weeks to provide a prediction, due to slow growth/response of the biological entity studies. Waiting for treatment until this prediction is finished is not feasible. While clinical machine learning models—once trained—take only seconds to minutes to run, they lack the capacity to prove causality in radiation effect. With explainable AI, this is attempted, but it will not surpass being hypothesis generating. However, when AI points to a potential biological process/mechanism, models of biology can show the causality of such processes through gene expression modulation. Retrospectively, biological assays can validate the predictive potential of specific biomarkers, as has been performed for organoid response testing.[38-41,124] If a model can predict patients' responses, single-cell multiomics can be used to show the biological processes involved. When such a process is observed and biologically validated by, for instance, knocking down the gene involved (Cinat et al.[44]), this process could also be assessed in patients. The stem cell genetic or biological process markers can be correlated with the response of general patients and their gene profile. An example could be the differential expression of cytokines involved in viral responses after irradiation, such as interferons (INFs)[44] between patients for tumor and normal tissue response. High levels of, e.g., INF-β may improve regeneration of normal tissue.[44] Say that some patients have a low, and others have a high INF response after irradiation; this could be linked to image "biomarker," clinical tumor characteristics, or GWAS data. Particularly, new image biomarkers from quantitative

imaging (e.g., DWI MRI or PET imaging with novel tracers) may be identified that need compelling rationale from biology to be introduced into the clinical workflow. Via this avenue, the biology causality can be subsequently related to clinical response data in larger datasets to develop effective bioinformatics and AI/machine learning models. An approach involving high-level data analysis, bioinformatics, and artificial intelligence (AI)/machine learning is required to combine the complex and large amount of data on omics and patients-related parameters. The big data collected for a comprehensive multiomics project requires combining different data processing and integration approaches to explore the quantitative aspects (correlation analysis, multivariate factorization, and molecular network learning). Dedicated machine learning approaches (e.g., multivariable statistics, random forest, and possibly novel methodologies to be developed) need to be adequately tuned to analyze the data. For example, by applying unsupervised clustering methods, multifactorial clinical (image) biomarkers can be linked to relevant biological markers. Next, a machine learning Bayesian approach may be promising, assuming that higher irradiation doses will produce a higher response and that different radiation qualities produce a different response. It is also essential to link the biological approaches with the clinical prediction model, as clinical prediction models are trained on patients receiving the current status-quo radiotherapy fractionation schedule and prescribed dose. This means that the risk classification of patients may be altered if a different radiotherapy regimen would have been given, while this cannot be tested in vivo, as we can only give the primary radiation once. This could be done with biological methods, such as organoids. To effectively personalize the radiation dose, a symbiotic collaboration between the disciplines is essential.

The potential of multiomics data analysis is yet to be unraveled; in consulting with the team mentioned above and based on the diversity and quality of the data, potentially different methodologies may be followed.[125] This should lead to models per tissue/tumor type that, e.g., predict patients' specific responses. Multiomics data from validation experiments must be included to test further and verify the models. In the near future, this may lead to improved patient-specific prediction of tumor and normal tissue response, allowing optimization of radiotherapy and combined treatments, resulting in optimal tumor control with the best possible quality of life.

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