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ADVANCES IN RADIOTHERAPY SPECIAL FEATURE: REVIEW ARTICLE

Personalized radiotherapy: concepts, biomarkers and trial design

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ABSTRACT

In the past decade, and pointing onwards to the immediate future, clinical radiotherapy has undergone considerable developments, essentially including technological advances to sculpt radiation delivery, the demonstration of the benefit of adding concomitant cytotoxic agents to radiotherapy for a range of tumour types and, intriguingly, the increasing integration of targeted therapeutics for biological optimization of radiation effects. Recent molecular and imaging insights into radiobiology will provide a unique opportunity for rational patient treatment, enabling the parallel design of next-generation trials that formally examine the therapeutic outcome of adding targeted drugs to radiation, together with the critically important assessment of radiation volume and dose-limiting treatment toxicities. In considering the use of systemic agents with presumed radiosensitizing activity, this may also include the identification of molecular, metabolic and imaging markers of treatment response and tolerability, and will need particular attention on patient eligibility. In addition to providing an overview of clinical biomarker studies relevant for personalized radiotherapy, this communication will highlight principles in addressing clinical evaluation of combined-modality-targeted therapeutics and radiation. The increasing number of translational studies that bridge large-scale omics sciences with quality-assured phenomics end points—given the imperative development of open-source data repositories to allow investigators the access to the complex data sets—will enable radiation oncology to continue to position itself with the highest level of evidence within existing clinical practice.

BIOLOGICAL CONCEPTS IN RADIATION ONCOLOGY

Radiation remains one of the most effective treatment modalities in cancer and has a central role in controlling localized disease. The principal therapeutic intent of exposing the tumour to ionising radiation is to produce irreversible DNA damage that will cause tumour cell death. In principle, if the radiation dose is high enough, all clonogenic cells in the target volume will be exterminated. Technological advances in radiation delivery have enabled development of physical high-precision treatment protocols to improve patient tolerability for dose escalation. Yet, as high-energy radiation fundamentally is a biological intervention, it may fail to eradicate all clonogenic cells of a heterogeneous tumour containing components that are resistant to the therapeutic damage, such as cells adapted to survive within a hypoxic microenvironment. The tangible reality of this failure is local or distant disease recurrence.

Combined-modality radiotherapy

In the past two decades, the benefit of chemoradiotherapy (CRT), *i.e.* the concomitant addition of a systemic cytotoxic

agent during the course of radiation, has been demonstrated for a range of locally advanced cancers.¹ Conceptually, CRT produces more complex difficult-to-repair DNA damage² and can increase the number of logs of tumour cells killed to improve local control. It is debated whether combined-modality treatment may also cause better systemic control. Because radiation has the ability to deliver forceful biological effects in a focused volume of tumour, it has been argued that a better systemic outcome may be achieved by the enhanced probability of eliminating the source that maintains the population of clonogenic cells.³

With new insights into the mechanistic biology of radiation responses, there is an increasing opportunity for rational integration of molecularly targeted agents in clinical radiotherapy in an effort to optimise effects. Recognizing that biological therapies frequently have modest single-agent activities, they may rather have the potential to intensify the cytotoxicity elicited by radiation-induced DNA damage or even CRT, either directly or by counteracting defence signalling mechanisms of the tumour.⁴ The landmark study

confirming significant improvement in post-radiotherapy survival outcome for patients with head-and-neck cancer concomitantly treated with the antiepidermal growth factor receptor (EGFR) antibody cetuximab⁵ was the first substantial proof of this concept, although the more recent randomized trial of adding cetuximab to the standard cisplatin-containing CRT in the experimental arm failed to improve outcome.⁶ Nevertheless, an escalating number of radiotherapy studies are reporting on the concomitant use of systemic-targeted agents.^{2,7}

However, the unrivalled efficacy of radiation in controlling local tumour manifestations is a reflection of a delivered dose that is commonly at the limit of normal tissue tolerance. Any adverse event that causes interruption in the radiation delivery is likely to have a negative impact on the probability of tumour control.⁸ In the context of combining a systemic drug with radiotherapy, overlapping and synergistic toxicity profiles may prevail.⁹ It is therefore acknowledged that the achievements in survival outcome resulting from the more efficacious therapies that include increasingly complex multimodal programmes are at the price of extended limits of treatment intensity and patient tolerance.

BIOMARKER-DRIVEN ADAPTIVE RADIOTHERAPY

Biological modelling is increasingly important in clinical radiotherapy (Figure 1). It is believed that it may ultimately assist in individualization of patient treatment by enabling the rational design of next-generation trials that evaluate the relevant clinical end points, which are efficacy and toxicity. In considering the use of systemic agents with presumed radiosensitizing activity, this evaluation may also include the identification of molecular, metabolic and imaging markers of treatment response and tolerability; however, this will need particular attention on patient eligibility. In the following, we will provide examples from recent biomarkers that have addressed prevailing radiobiological concepts and further highlight principles of the clinical evaluation of combined-modality radiotherapy with targeted therapeutics.

Molecular biomarkers in oncology

For the use of systemic single-agent-targeted therapeutics, several predictive biomarkers based on gene mutations are integrated into clinical routine. Examples of well-established biomarkers are tumour KRAS/BRAF/NRAS mutation status for treatment of metastatic colorectal cancer with antiEGFR antibodies and BRAF V600 mutations in metastatic melanoma for targeted treatment of

Figure 1. Biological modelling is the backbone of study design in personalized radiotherapy. High-throughput omics and functional imaging technologies are applied to determine tumour and circulating molecular, metabolic and imaging signatures descriptive for the patient's tumour and normal tissue constitution. This information is utilized to individualize the combined-modality radiotherapy protocol to each specific case. Curated phenomic data from clinical end point assessments (treatment toxicity and efficacy) are integrated together with the biological data in open-source repositories to provide investigators the access to the data sets within global networks. As a toolbox for data mining across a range of technology platforms and based on rational study design, the ultimate objective is to develop knowledge-driven decision support systems to guide clinical practice by the learning models that are generated. Images are from our own work or purchased from a commercial stock photo agency.

positive cases. However, this paradigm cannot easily be transferred to combined-modality radiotherapy. Although considerable progress has been made, the current knowledge of the molecular basis of tumour cells' sensitivity to radiation in combination with radiosensitizing systemic agents remains insufficient for routine implementation into molecularly stratified radiotherapy.

Most current molecular biomarker approaches are based on gene point mutations, amplifications or translocations.¹⁰ It is appreciated that such biomarkers may not be sufficient in selecting the optimal targeted therapy, as multiple molecular changes, rather than a single gene modification, commonly lead to the aberrant activity of signalling cascades that promotes tumour progression. Moreover, technological advances have paralleled the new biological knowledge and led to development and application of a range of high-throughput omics technologies, such as genomics, proteomics, metabolomics and kinomics, capable of producing molecular signatures of tumour tissue, serum, plasma and other body fluids.^{11–15} Such profiling may unravel the complex signalling circuits of individual tumours and appears attractive for the determination of predictive biomarkers.

However, recognizing the biological heterogeneity of tumours, there is a potential for sampling error when a small biopsy is taken from a much larger tumour. This challenge is addressed by an increasing number of investigators, particularly for reliable diagnosis of the typically multifocal prostate cancer, where protocols of image fusion targeted biopsy sampling have been implemented at many centres.^{16–18} We anticipate this approach to be introduced on a wider basis as the required technologies develop and become accessible in routine hospital practice.

Imaging biomarkers in oncology

The past decade's expansion of medical imaging technologies in radiation oncology has led to their development from solely being diagnostic tools into a role in treatment individualization. Even tumours of identical histological types demonstrate considerable biological heterogeneity reflecting ample variations in the individual tumour's constitution, which is a result of local variations in cell proliferation and death, metabolic activity and vascular structure and is accompanied by diversities in oxygenation status, pH and drug delivery that will directly affect therapeutic efficiency. This extraordinary spatial and temporal intratumoral heterogeneity at the gene, protein, cell, metabolism and microenvironmental levels represents a major limiting factor for biopsy-based molecular assays; by contrast, non-invasive imaging technologies comprise a huge potential for depicting, in a three dimensional fashion, the intratumoral biology.¹⁹ Although one must recognize the limitation in spatial resolution on clinical imaging scanners, where reliable visualization at the single-cell level is not (yet) attainable, in recent years, the identification of effective imaging biomarkers have evolved into an area of immense investigations.

Several imaging biomarkers have been identified by applying functional MRI and positron emission tomography (PET), enabling characterization of a range of physiological and biological

tissue functions, and which represent a promising avenue for further individualization of cancer patient management.^{20–23} At present, no imaging biomarker addressing optimal utilization of targeted therapeutics and radiation has been established in clinical routine practice but a range of emerging candidate biomarkers have been identified. Examples include parameters derived from post-processing analysis of images obtained by ¹⁸F-fluorodeoxyglucose PET, ¹⁸F-fluoromisonidazole PET, ¹⁸F-fluoroazomycin-arabinoide PET, diffusion-weighted MRI, dynamic contrast-enhanced (DCE) MRI, blood oxygen level-dependent MRI and MR spectroscopy.^{24–28} It is envisaged that imaging technologies have great potential to reduce the number of patients required to test novel targeted agents by predicting or identifying non-responders early on and, hence, enriching the clinical study population with patients more likely to benefit.²⁹

A major obstacle hindering the clinical use of imaging biomarkers has been the wide variation in which imaging data have been collected and analysed, such as the absence of standardized and independently validated software for both data acquisition and post-processing data analysis. Study populations have often been small and from single institutions, and technique reproducibility has generally not been investigated.²⁴ Before routine clinical integration of novel imaging biomarkers addressing the combination of targeted therapeutics and radiation, its potential will require evaluation in well-designed prospective trials classifying patients into distinct groups based on standardized procedures.

Molecular biomarkers of tumour hypoxia

Since tumour hypoxia is recognized as a main factor in radiation resistance, metastasis development and poor disease survival, major efforts have focused on determining tumour signatures for reliable patient stratification to hypoxia-modifying agents in combination with radiotherapy. In head-and-neck cancer, specific gene hypoxia signatures distinguished between high- and low-hypoxic tumour xenografts and further enabled prediction of post-treatment patient outcome.^{30,31} Similar signatures have also been retrieved in other tumour entities.^{32,33} Moreover, in head-and-neck cancer, high tumour staining of the hypoxia marker pimonidazole correlated with inferior locoregional control,³⁴ and in tumour samples from patients with prostate cancer, expression of genes involved in proliferation, DNA damage repair and hypoxia correlated with both pimonidazole staining and a particularly aggressive phenotype.³⁵ Interestingly, a recent multi-institutional study identified and further validated an interactive influence of tumour hypoxia together with genomic instability on biochemical relapse following radical radiotherapy or prostatectomy in intermediate-risk patients.³⁶ Also, hypoxia-induced microRNA-210 expression has been observed in a range of experimental tumour models and patient tumour samples.^{37–39}

Compared to markers based on tumour biopsies, blood-based factors (also termed liquid or fluid biopsies) primarily benefit from being readily accessible. In patients with head-and-neck cancer, high plasma osteopontin concentration showed correlation to tumour hypoxia and unfavourable outcome after radiotherapy.⁴⁰ Another head-and-neck cancer study evinced that

certain nutritional antioxidants and oxidative stress markers were associated with post-radiotherapy survival outcome.⁴¹

Combining multidimensional information of tumour hypoxia

One example of a multidimensional approach was illustrated by the combined analysis of gene expression and the amplitude parameter *A* derived from Brix kinetic modelling of DCE MRI, which detected a hypoxic, poor-prognostic tumour phenotype in patients with cervical cancer scheduled for CRT.⁴² Patients with head-and-neck cancer with high baseline tumour ¹⁸F-fluoromisonidazole uptake are known to have high risk of locoregional failure;⁴³ however, there is evidence that residual tumour hypoxia, as measured both in pre-clinical models and by ¹⁸F-fluoromisonidazole or ¹⁸F-fluoroazomycin-arabinozide PET in several patient cohorts, early in the course of fractionated radiotherapy for head-and-neck cancer both has more potential for therapy adaptation and is a stronger predictor of outcome than baseline hypoxia.^{44–47} Moreover, in this patient group, hypoxia-targeted interventions (e.g. nimorazole or nicotinamide combined with carbogen breathing) have shown promising results,^{48–50} although the randomized trial of adding tirapazamine to cisplatin-containing CRT in the experimental arm failed to improve overall survival.⁵¹ In considering the conduct of future combined-modality studies, this will increasingly highlight the dynamic nature of the tumour biology, for example, by including serial biomarker assessments during the course of fractionated radiotherapy to enable biological adaptation to changing tumour vulnerabilities, which will be further discussed below.

Following considerable technical progress, quantification of image features is now possible by the application of leading-edge software. The high-throughput extraction and analysis of large amounts of advanced features from radiographical images are referred to as radiomics.^{52,53} An exciting potential lies within the ability to build descriptive and predictive models relating image features to phenotypes and converting this information into mineable data through a high-accuracy pipeline process of image acquisition and reconstruction, segmentation, feature extraction, database sharing and *ad hoc* informatic analysis.⁵³ Inherent challenges of standardization of imaging biomarker techniques and analysis methods are being addressed through a number of ongoing initiatives. Some examples are the Quantitative Imaging Biomarker Alliance,⁵⁴ the Quantitative Imaging Network,⁵⁵ the American College of Radiology Imaging Network,⁵⁶ the Quantitative Imaging in Cancer—Connecting Cellular Processes with Therapy Consortium⁵⁷ and the European Institute for Biomedical Imaging Research.⁵⁸

Biomarkers of DNA damage and repair

Besides hypoxia factors, biomarkers of tumour sensitivity to radiation and, hence, of possible therapy targets include DNA damage markers and in particular, proteins involved in repair of DNA double-strand breaks, the most lethal of DNA lesions. A commonly investigated factor is γ H2AX, a histone protein that is phosphorylated following induction of DNA double-strand damage, forming foci around the breaks. Accumulating evidence indicates that the number of residual foci in tumours following

radiation correlates with radiosensitivity.⁵⁹ Both in patients with cervical cancer and head-and-neck cancer xenograft models, the presence of γ H2AX foci colocalized with hypoxic tumour areas,^{60,61} however, the ability of γ H2AX to predict treatment failure has shown inconsistent results. Another DNA damage repair marker investigated in clinical studies is the repair protein Ku80.^{62,63} Conceptually, such markers may be particularly useful for stratifying patients to combined-modality radiotherapy with agents that specifically inhibit tumour DNA repair processes. For instance, inhibiting the DNA repair enzyme poly(ADP-ribose) polymerase has resulted in effective tumour cell radiosensitization both *in vitro* and *in vivo* as well as increased radiosensitivity in repair-deficient hypoxic cells.⁶⁴

Biomarkers of radiation-induced adverse effects

The possible determination of DNA damage repair mechanisms in normal tissues appears explicitly interesting for the purpose of identifying patients with high risk of developing adverse responses to radiation. However, at present, optimized establishment of standardized DNA damage repair assays and their prospective assessment in large patient cohorts remain incomplete.^{59,65}

Interestingly, a number of single nucleotide polymorphisms (*i.e.* germline gene variations) of DNA repair genes have been acknowledged as candidate biomarkers of susceptibility for untoward radiation effects in both short-term and long-term perspectives.^{66,67} Recently, a genome-wide association study in patients with prostate cancer given external radiotherapy identified TANC1 as susceptibility locus for late radiation-induced damage.⁶⁸ Other studies have indicated low levels of radiation-induced apoptotic lymphocytes in patients (with miscellaneous cancers) experiencing severe late radiation-induced toxicity⁶⁹ or significant association between high mitochondrial DNA variation in skin fibroblasts and severe radiation-induced deep-tissue fibrosis in individuals treated for nasopharyngeal cancer.⁷⁰

In the context of treatment toxicity, analysis of circulating factors would be practicable but still remains to be implemented in routine practice. Several studies have investigated the feasibility. In predicting lung toxicity (pneumonitis and pulmonary fibrosis), which is major dose-limiting events in thoracic radiotherapy, candidate circulating factors such as transforming growth factor- β 1 and interleukins 1 and 6 have been identified.⁷¹ In breast cancer, multimodal protocols consisting of chemotherapy, radiotherapy and for a number of patients also antiHER2 therapy, pose significant risk of cardiac toxicity. In this context, the current practice of serial cardiac function monitoring by echocardiography is regarded as suboptimal. At present, the most promising circulating biomarkers are cardiac troponins and natriuretic peptides, which have demonstrated ability to reflect cardiac dysfunction in clinically asymptomatic patients.⁷²

REQUIRE—a biomarker study of radiotherapy toxicity

In light of the many patients that experience long-term adverse effects that may severely affect quality of life,⁷³ more systematic strategies must be solicited, most importantly for the benefit of the individual but also in respect of the costs this generates for

health services. Recognizing the deficiency of uniform reporting systems for long-term sequelae as well as clinically validated biomarkers for such effects, it has been proposed that establishment of predictive large-scale models may have the potential to translate into improvement of cancer survivors' quality of life and also limit healthcare costs.⁷⁴ The REQUITE project,⁷⁵ initiated by the Radiogenomics Consortium,⁷⁶ is a multicentre study for an anticipated cohort of 5300 patients with breast, lung or prostate cancer, from which blood samples, epidemiology data and treatment information as well as longitudinal data on side-effects and quality of life will be collected and stored in centralized biobanks and databases.⁷⁷ Among the project aims are the validation of already published biomarkers of radiosensitivity, the development of clinical predictors of radiotherapy toxicity and the design of interventional trials to alleviate long-term adverse effects. The successful execution of projects such as REQUITE will depend on the use of standardized forms and questionnaires as well as acquisition and analysis of high-quality tissue and blood samples utilizing standardized operating protocols, where data are being stored in large repositories to enable cross-centre data evaluation.

DECISION-SUPPORTIVE TOOLS IN RADIATION ONCOLOGY

To facilitate decision-making and enable improved individualization and resource optimization in contemporary radiation oncology, validated multifactorial prediction models of expected treatment outcome are highly desired, often referred to as clinical decision support system (DSS) models.⁷⁸ A DSS should integrate multiple features related to patients' disease, such as clinical, imaging and molecular factors, to achieve high-accuracy prediction of local tumour response, survival probability and treatment toxicity risk, and ideally also quality of life and fiscal issues of cost-effectiveness (Figure 1).

The DSS development may also motivate research on specific risk groups.⁷⁹ At present, treatment individualization is made on the basis of general guidelines resulting from large randomized trials only taking the relatively indiscriminate features such as tumour stage and physical condition of the patient into account. These guidelines are developed for groups of patients and will inevitably cause too aggressive treatment in some patients and insufficient treatment in others, leading to major expenses and detriments both for individuals and at the societal level. Predictive models based on features of the individual patient are likely to complement existing guidelines, enabling more quantitative decision-making.

Importantly, DSS studies may incorporate rapid learning models as a framework where previously collected clinical data are used to validate the DSS. Such knowledge-driven algorithms extract information from routine clinical data from each patient rather than solely depending on evidence from clinical trials.^{80–82} One example is the rapid learning network project that resulted in the DSS termed www.predictcancer.org.⁸³ By entering various clinical and imaging input variables into the model, output variables such as treatment response and overall survival are predicted in rectal, lung and head-and-neck cancer. The quantity and heterogeneity of data necessary for developing high-quality rapid learning models will

benefit from multi-institutional pooling of data, instead of only relying on local data capture.⁷⁸

One recent example of a rapid learning model extracting routine care data demonstrated the ability to predict overall survival in lung cancer. By using patient and tumour features, prognostic groups in whom therapy could be individualized were identified based on the predicted outcomes.⁸⁴ In another lung cancer cohort, prospectively collected data were utilized in a DSS to predict 2-year survival, dyspnoea and dysphagia outcomes following CRT. Comparison of model outcome and predictions performed by experienced radiation oncologists and guideline-based recommendations favoured the model-based identification of risk groups.⁸⁵

In the setting of combined-modality radiotherapy with targeted therapeutics, and with the advent of more sophisticated imaging and treatment-planning techniques, the radiation target volumes may shrink. However, the resulting normal tissue complications may still equal those observed with traditional extensive-field radiotherapy. Even when standard radiation dose–volume objectives are satisfactory and, consequently, the radiotherapy treatment plan is considered safe by conventional criteria, long-term toxicity may be significant. Recently, a multiple end point-interactive DSS was developed in order to provide quantitative risk estimates to supplement the clinical decision when comparing different radiotherapy alternatives.⁸⁶

The key for developing accurate and validated prediction models is standardization, both in acquisition of multiparametric data reflecting molecular- and imaging-based as well as treatment information, and also in patient preferences. Although DSS models have the potential to change clinical practice, their application is at present hampered by the lack of impact studies. Showing their theoretical benefit, however, will likely stimulate the conduct of such studies. Additionally, it will pave the way for more advanced models that also incorporate omics data. Recognizing the superior performance by several models, it is contended that the sole reliance on doctors' opinions and general guidelines may even prove unethical in the future.⁸⁵

LOCALLY ADVANCED RECTAL CANCER—A MODEL-OF-CONCEPT

In locally advanced rectal cancer (LARC), randomized studies have shown the superiority of neoadjuvant CRT in conjunction with resection of the residual tumour,⁸⁷ resulting in local recurrence rates well below 10%.⁸⁸ Yet, there is compelling evidence that long-term survival benefit is contingent on considerable or factual complete tumour response,⁸⁹ supporting the notion that elimination of tumour clonogens is essential for favourable therapeutic results. Specifically, a substantial number of patients with LARC, reported to be 30–40% of cases in recent studies,^{90,91} will experience metastatic progression.

In pelvic CRT, the development of acute intestinal toxicity, clinically presenting as significant diarrhoea and associated metabolic disturbances, represents the major limitation to delivering the prescribed therapy. Toxicity is commonly experienced towards the treatment completion and is usually transient.

However, severe enteritis is strongly associated with interruption or pre-mature CRT cessation and, as a result, an adverse patient outcome.⁸

In the everyday clinical practice, it is recognized that rectal cancer presents with a high degree of biological heterogeneity.⁹² A prevailing hypothesis is that in the primary tumour, expansion of distinct subclones under selection pressure within hypoxic microenvironmental niches gives rise to such heterogeneity.^{93,94} Consequently, critical understanding of the biological diversity is needed to improve existing therapies both for the initial eradication of primary tumour clonogens and, importantly, prevention of metastatic progression.

Decision support system models in locally advanced rectal cancer

Several multi-institutional randomized trials have addressed a number of questions regarding combined-modality treatment of LARC, including the optimal integration of radiation and chemotherapy, the sequencing of modalities as well as radiation dose fractionation. However, tools or measures to optimally choose among several potential treatments at the individual patient level remain scarce. This challenge will be even more complex with the integration of targeted therapeutics. Some initial studies have addressed this issue by the use of nomograms. For instance, multivariate nomograms were developed by pooling a range of clinical, therapeutic and histopathological variables collected in five major European trials and were shown to predict risk of local recurrence, distant metastasis and overall survival. Additionally, the nomograms provided decision support for administration of postoperative chemotherapy to an identified high-risk group and suggested individualization of follow-up based on predicted recurrence risk.⁹⁵

The potential of imaging to provide relevant predictive information was shown by a nomogram comprising sequential ¹⁸F-fluorodeoxyglucose PET/CT data from 953 patients with LARC receiving CRT. The nomogram predicted histopathological complete response following CRT with relatively high accuracy and was suggested as a DSS to more individualized surgical approaches of LARC.⁹⁶

The lack of other sophisticated nomograms in LARC may reflect the prevailing non-individualized standard of care. Currently, local treatment of LARC comprises either short-course radiation or long-course fluoropyrimidine-based CRT followed by surgery. However, sequencing of radiation and chemotherapy as well as new surgical approaches and integration of targeted therapeutics as an additional component of CRT will offer opportunities for more individualized treatment.⁹⁷ Recently, an umbrella protocol for standardized data collection was established, aiming at uniform procedures to obtain a consistent data set that may enable prediction of treatment efficacy and toxicity within the framework of a DSS.⁷⁹

CRITICAL CONSIDERATIONS IN TRIAL DESIGN

By contrast to studies that examine effects and toxicities of single-agent treatment, whether this is radiotherapy or drug protocol, the combination of a new systemic compound with radiation is a more

complex trial context that demands special consideration of study design and end points that reflect both radiation effect and potential independent and overlapping toxicities of the two modalities. In considering the use of biologically targeted radiosensitizing agents in combined-modality treatment protocols, this requires particular attention to the definition of patient eligibility and radiation dose–volume relationships in evaluating normal tissue toxicities. Additionally, the design may implement tracks to identify markers as surrogate end points of treatment efficacy and tolerability.

Of fundamental consideration in the design of radiotherapy trials is the quality of standard of care to patients. Poor-quality radiotherapy without proper attention to critical factors such as target coverage, sparing of organs at risk and internal motion during treatment has the potential to dominate outcome and mask the benefit from a systemic agent with presumed radiosensitizing activity. Recent reviews of radiotherapy delivery within multicentre trials have shown that failure rates were significantly higher after protocol-non-compliant radiotherapy and have further highlighted the need for comparable quality assurance procedures within intergroup trial collaborations.^{98,99}

Below, some further principles relating to studies combining new drugs with radiotherapy will be discussed.

Pre-clinical evidence

In recent years, laboratory studies have identified a wealth of potential radiosensitizing compounds; however, translating such findings into new combined-modality radiotherapy regimens requires thorough pre-clinical evaluation. A candidate substance should be tested using appropriate assays and relevant experimental models in order to address several end points. Among the most important issues to elucidate are whether the drug target is selective for the radiosensitization of tumour over normal cells¹⁰⁰ and, if feasible, the influence of various micro-environmental conditions on the radiosensitizing properties of the candidate drug.¹⁰¹ Consensus recommendations on how to translate novel radiosensitizing agents from pre-clinical investigations into early-phase clinical assessment have recently been published.^{102,103}

With regard to possible normal tissue toxicities in particular, various experimental models for correlative mechanistic analyses may be considered⁹ but should be relevant to the clinical scenario and ideally consider both acute and late adverse effects. A technological approach that has been increasingly commented on recently is human microphysiological systems, which are microscale “on-chip” models of human multiorgan systems;¹⁰⁴ however, the potential of such three dimensional organ platforms in prediction of tissue perturbations to external stimuli needs extensive developmental experimentation.^{105,106} It is also argued that the zebrafish embryo system may be a tool for pre-clinical toxicity assessment.¹⁰⁷ Relevant acute and late normal tissue effects of combined-modality therapy have been demonstrated in mice as a model organism.¹⁰⁸

Patient safety

A combined-modality therapy study may be designed to demonstrate a number of key questions; still, one of the main objectives

will be to assess whether the combination of the systemic drug and radiation is safe and tolerable. In an intensified curative treatment schedule at the limits of normal tissue tolerance, the increased risk of interruption or premature cessation of the radiotherapy and, hence, deleterious effects on patient outcome must be specifically addressed in the study design.

In a study setting of evaluating tolerability of a first-in-human combination of radiation with a systemic therapeutic, which is applicable also for the combination with a molecularly targeted agent with toxicity profile that is independent from the organ-confined cytotoxic effect caused by radiation of the specific target volume, only patients who are not candidates for any curative radiotherapy protocol should be regarded as eligible.⁷ As a general principle, committees for medical and health research ethics will waive approval of first-in-human experimental therapeutic approaches in patients with curative intention of standard treatment.

However, adverse effects from combined-modality radiotherapy will commonly be different in a palliative regimen compared with an intensified curative radiation schedule for earlier-stage disease.⁷ Firstly, the combination of radiation with a systemic drug with radiosensitizing activity must take into account both intensity and duration of the toxicity profiles that have been observed within a palliative regimen. With curative intent, where the biologically equivalent radiation dose commonly is higher, the study may therefore initially adopt a Phase I design that implements escalation from a low starting dose of the investigational agent prior to a subsequent efficacy investigation following the determination of a recommended Phase II dose. Secondly, the detriment of an interruption in the radiation delivery within an intensified curative combined-modality protocol that encounters toxicity at the limits of normal tissue tolerance needs particular attention, although such studies may be conducted in patient groups with historically poor treatment outcomes, for example, locally advanced lung cancer or pancreatic cancer.¹⁰² A poor-outcome setting may also provide the best chance of observing a benefit from the new intervention, if it exists. Finally, it is important to keep in mind that late radiation toxicity is often not explicitly considered in first-in-human trials, which is a critical limitation of early-phase combined-modality studies.

It is contended that quantification of treatment toxicity inherently is much more complex than quantification of treatment efficacy because of the huge variation in severity of adverse events among individuals treated for cancer. However, the National Cancer Institute's Common Terminology Criteria of Adverse Events (CTCAE) was established as a system for recording toxic effects with all types of cancer therapy and to uniform severity scaling. Close attention was paid to the boundary between grade 2 and grade 3, demarcating a clearly higher level of severity.¹⁰⁹ The CTCAE grade 3 and 4 toxicities reflect injury of grave or life-threatening severity, respectively, which implicates that such events often are used to trigger dose reductions or other therapy adjustments in addition to intensified supportive care intervention, usually involving hospital admission. Importantly, patient-reported outcomes, typically in terms of standardized quality of life questionnaires or similar

patient-related measures, are crucial for complete evaluation of patient safety, for example, as seen developed within the REQUITE project.⁷⁵

In summary, it is of utmost importance that protocols in personalized radiotherapy have a prospective design to enable uniform recording of toxicity score as a critical end point. In general, our understanding of underlying mechanisms of treatment toxicity lags far behind that of tumour response,¹¹⁰ a realization that strengthens the necessity of applying validated scientific methodologies at every step of the assessment and tentative biological understanding of normal tissue response to treatment exposure.

Radiotherapy technique

By contrast to systemic therapy protocols where disease location and treatment tolerability commonly are independent factors, in radiotherapy the site of the target lesion determines normal tissue toxicities. Hence, full interpretation of toxicity data in combined-modality therapies requires clear specification of the radiation target volume as well as detailed description of radiation dose–volume dependencies within the protocol, as mentioned above and further discussed below. This will also enable the recognition of separate adverse effects from the radiation and the systemic agent.

Because treatment toxicity in radiotherapy typically reflects the size of the target volume and the dose distribution within it,^{8,111} in reporting the radiation technique the description should include precise definitions of the specific target volumes, tumour dose and dose fractionation, overall treatment time and dose variations within relevant organs at risk.⁷ The protocol may also specify a detailed description of dose–volume constraints according to the QUANTEC recommendations.¹¹²

Consequently, when the radiotherapy is delivered to appropriate target volumes as determined by state-of-the-art imaging-based treatment planning, normal tissue dose–volume effects can be quantified from the treatment-planning data set. This may enable estimation of radiation-induced adverse events specifically, which is particularly important in studies of treatment intensification, such as radiation dose escalation, the therapeutic enhancement of alternative radiation-drug scheduling or the use of radiosensitizing drugs.⁹

The Pelvic Radiation and Vorinostat study for symptom palliation in advanced bowel cancer^{113,114} was an example of an investigation into the safety of using a molecularly targeted drug in clinical radiotherapy. The study was designed to determine tolerability when combining vorinostat with radiation to pelvic target volumes, in which acute bowel toxicity is frequently encountered by the radiation exposure alone. Because common side-effects of vorinostat single-agent therapy include intestinal toxicities,¹¹⁵ in evaluating the study data, it could therefore be difficult to decide whether or not an adverse event occurring during treatment was greater than might be expected for either of the therapeutic components, or in other words, to determine whether the reported CTCAE grade 3 toxicities should be considered as having been caused specifically by the systemic agent.

The study adopted a conventional Phase I expansion cohort design¹¹⁶ undertaken in sequential patient cohorts exposed to escalating dose levels of the investigational drug (Dose-levels 1–4) administered concomitantly with the pelvic palliative radiotherapy. The reported CTCAE grade 3 toxicities (*i.e.* dose-limiting toxicities) were intestinal and related adverse events.¹¹³ The study patients had radiation treatment-planning imaging scans visualizing the entire abdominal and pelvic cavities, enabling contouring of all individual loops of the small bowel on the scans and therefore the generation of total small bowel dose–volume histograms. Hence, the relative small bowel volume receiving the fully prescribed radiation dose could be quantified.¹¹⁴ One patient at Dose-level 3 of vorinostat reported CTCAE grade 3 intestinal toxicity. This might have been an adverse radiation dose–volume effect rather than a toxic effect of the investigational drug since as much as 40% of the total small bowel volume received the total radiation dose. Study patients reporting CTCAE grade 3 toxicities at the highest dose level of vorinostat (Dose-level 4) displayed substantially smaller relative volumes of small bowel exposed to high radiation dose, and their radiation dose–volume records were essentially indistinguishable from estimates in patients without grade 3 adverse events. Hence, in this study, there seemed to be a threshold volume of irradiated small bowel that distinguished between patients with high and low risk of severe bowel toxicity. This observation emphasizes that the description of detailed radiation dose–volume constraints within the treatment protocol will facilitate study data interpretation when overlapping toxicities between a systemic compound and radiation may appear.⁷

Further in line with these notions, iatrogenic complications, whether acute or chronic morbidity, that are associated with current treatment protocols may call for treatment intensity de-escalation studies in tumour entities with very low failure rates. Human papillomavirus-associated oropharyngeal squamous cell carcinoma is an example where a number of randomized de-escalation studies are ongoing.¹¹⁷ Study strategies of alleviating treatment toxicities either comprise the replacement of cisplatin with cetuximab in the CRT regimen, induction chemotherapy with the aim of lowering the subsequent radiotherapy dose in good-responding cases or prescription of de-escalation protocols following minimal-invasive surgery of early-stage disease.

Biomarker end points

In personalized radiotherapy trials, inclusion of surrogate end points should be encouraged where patients eligible for a correlative biomarker side-study may sign a separate consent regarding the actual procedures. Practical issues may relate to the availability and retrieval of high-quality research material within the context of a therapy study. One example is biopsy sampling from deep-seated tumours, which may be hazardous and, if associated with complications, will delay the commencement of therapy. The study can be designed to reduce the risk of untoward events if patients receive the study drug for a period before starting the radiotherapy course, for example during the treatment-planning period.¹⁰²

The intense focus on molecularly targeted compounds has been facilitated by the tremendous developments in high-throughput comprehensive technologies for molecular profiling. In selecting cancer patients to molecularly targeted agents, when a validated

biomarker exists, prevailing strategies are mainly based on detection of tumour gene aberrations.¹¹⁸ However, such biomarkers may not be sufficient for the purpose of the more complex combined-modality protocols or because multiple gene aberrations, which in solid tumours often are the case rather than a single driving gene modification, may affect a wide range of components of the signalling network and the potential tumour responsiveness accordingly. Of further note, the plasticity of microenvironmental changes in hypoxic tumours will also contribute to the diversity in signalling activity. Consequently, methodologies comprising the resultant condition of interacting signalling effects may be advantageous to identify relevant biomarkers in the context of combined-modality treatment. Kinase substrate array technologies are tools for global profiling of kinase activities in tissue samples without prior knowledge of which signalling pathways are active.¹¹⁹ Using such arrays, each tumour sample will generate an individual phospho-substrate signature, theoretically portraying the state of composite information flow through signalling cascades as biomarkers for a targeted approach of enhancing radiation responsiveness.^{120,121}

The systematic use of multimodality functional imaging strategies (*e.g.* markers of tumour metabolism, hypoxia and angiogenesis) will undoubtedly contribute in furthering understanding of responses to combined-modality treatment protocols with radiation and targeted therapeutics and particularly, in identification of surrogate imaging biomarkers for outcome. The successful advancement of imaging biomarkers will depend on their intelligent investigation; importantly, by exploiting the possibility to easily and non-invasively perform serial assessments of whole-tumour phenotypes before and on-treatment and, as a next step, by identifying specific biomarkers for each class of treatment combination rather than generic markers.²⁸ Integration of imaging biomarkers in personalized radiotherapy trials will require the confidence that these markers are technically valid, that a measured change indeed reflects the desired change in the underlying tumour biology and that the actual markers can be applied at multiple cancer centres in a robust (by being valid across vendors and observers), consistent, ethical and cost-effective way.¹²² Herein, the efforts within co-operative clinical trial groups are crucial.

Regarding adverse treatment effects, it may frequently be challenging to evaluate separate toxicities of the systemic component within the overall treatment toxicity profile dominated by radiation adverse effects. Therefore, drug-exposed, non-irradiated surrogate tissue may be sampled from patients for the identification of toxicity biomarkers of the systemic agent that are not simultaneously manifesting perturbations caused by radiation.¹²³ Ideally, the normal tissue that best manifests the actual clinical phenotype should be analysed for causative mechanisms of treatment severity at the individual level but may not be readily available. Therefore, at the study population level, a surrogate normal tissue is commonly accepted as being more feasible for correlative mechanistic analysis.⁹

As an example, in neoadjuvant treatment of LARC, serum protein profiles specific for mucosal inflammation (*i.e.* enteritis) might be markers of intestinal adverse effects since acute radiation enteropathy is strongly associated with activation of

mucosal inflammatory cytokines,^{124,125} also in experimental models.¹²⁶ One approach might be to utilize multiplex protein technology to discover changes in serum proteins throughout the neoadjuvant treatment course and correlate alterations in circulating levels of specific mediators with the adversity of diarrhoea.⁹ Within the context of a prospective study, multiplex data of this kind would bring new biological insight into the pathophysiology of bowel toxicity following cytotoxic therapy.¹²⁷

Of general note, mechanism-based prediction of treatment toxicity is a new research avenue in oncology, and its extrapolation into the discipline of combined-modality therapy adds another layer of complexity.⁹ Recognizing the multiplicity of molecular targets that are altered by radiation, biomarker-driven studies may be challenging to design, particularly in the context of predicting potential interactions between systemic agents and radiation during concomitant or sequential therapy. One example from routine clinical practice is patients with advanced cancer where systemic drugs enable longer periods of disease stabilization and also altered pattern of failure. An increasing number of cases will require palliative radiotherapy at the time of coadministration of a systemic drug, and the potential for normal tissue toxicity is often still unclear.

PERSONALIZED RADIOTHERAPY—PERSPECTIVES AND CHALLENGES

In recent decades, radiation oncology has experienced huge advances both in anatomical imaging of treatment targets and in treatment delivery technology. However, it is unlikely that further advances in physical targeting and fractionation alone will result in significant improvement in survival outcome among patients with locally advanced and, indeed, metastatic disease.¹²⁸ The next developmental step will more likely make use of the evolving wealth of biological knowledge. One example is to attack hypoxic tumours by targeted interference with hypoxia-activated signalling pathways and metabolic changes. The emerging concept of radiotherapy as an adjuvant for immunotherapy in oligometastatic disease is another intriguing example.^{100,129} These and other biology-based strategies may contribute to selectively sensitizing the tumour for optimization of tumour control, to reducing normal tissue injury from radiation damage and even to modulating the immune system for improved management of locally advanced and possibly also metastatic disease.¹⁰⁰

However, a number of challenges are still to be solved in further investigations of biologically targeted agents in combination with radiotherapy. Such initiatives are regrettably hampered by the approach to drug development that focuses almost exclusively on demonstrating efficacy in the metastatic disease setting, which inadvertently will evade the opportunity of identifying agents that would provide their strongest clinical impact by making the tumour more vulnerable to radiation. Both the expense of developing new drugs as sole radiosensitizers, which are not to be used at the highest tolerated doses but at lower biological doses and in addition for a relatively short treatment period, and the potential that radiation-induced toxicity might slow down approval may restrict new investment by the pharmaceutical industry or their collaboration with academic investigators. The unfortunate consequence is that the clinical potential of a novel drug is not fully acquired because the patient population that could benefit will not be found.¹

Clinical studies that enable bridging of omics (molecular, metabolic and imaging data) with phenomics end points, those being intended (treatment efficacy) or adverse (treatment toxicity) responses, are critical for the further advance of contemporary cancer therapy. A controversial issue in this regard may be the development and protection of large-scale data acquisitions and open-source data repositories and how to provide investigators with access to the complex data sets generated by multidimensional analyses. Additionally, it is critical that the underlying clinical data are correctly annotated and quality assured. As outlined by Figure 1, in order to achieve the desired standard of integration of all existing data that reside in widely located sources within the industrial, academic, regulatory and clinical practice sectors, there is a requirement for open-source data repositories with the aim of facilitating the formation of global networks in biological disciplines.¹³⁰ As templates for future studies, such major tool sets for data mining, often across different technology platforms, will undoubtedly be beneficial as a system to support collaborative projects in general and particularly as public resources of highly curated data to advance the field of patient safety in cancer therapy.¹³¹

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