Chapter 1
Introduction to Molecular Targeted Radiosensitizers: Opportunities and Challenges

Henning Willers and Iris Eke

Abstract The practice of radiation oncology is currently primarily based on precise technical delivery of highly conformal, image-guided radiation treatments. The precision medicine revolution has provided radiation oncologists with tremendous opportunities to enhance the anti-tumor effects of radiation therapy, potentially with less normal tissue toxicity than traditional chemotherapeutic radiosensitizers. However, a large body of preclinical research and clinical investigations on radiosensitizers has not yet translated into any meaningful number of FDA-approved combinations of radiation with targeted radiosensitizers ± chemotherapy. There exist distinct challenges to clinical translation of radiation/drug combinations that the field is only beginning to appreciate. These considerations have served as motivation for this book, which provides a comprehensive review by experts in the field of key preclinical research components required to identify effective and safe (chemo-)radiosensitizing drugs. Readers will be provided with a detailed and timely insight into the framework of targeted radiosensitizer research coupled with recent developments in immuno-oncology. Ultimately, this book will support the identification of appropriately validated and biomarker-directed targeted drug/radiation combinations that will have a higher likelihood than in the past to be incorporated into standard management of human cancers. These developments, coupled with the increasing technical power of radiation therapy to safely increase local control for many solid tumors, are expected to improve survival outcomes and cure rates for our patients.

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1 Opportunities for Targeted Radiosensitizers

Radiation therapy is an important treatment modality that is given to over 50% of cancer patients at some time during the course of their disease (Bristow et al. 2018). The goal of curative radiation therapy is to sterilize all cancer stem cells (CSC) or CSC-like cells that could give rise to a local tumor recurrence while limiting injury to normal tissues around the tumor and to the patient (Willers et al. 2019). Curative radiation is often combined with surgery or/and chemotherapy depending on cancer type, tumor stage, and other factors. In clinical settings where cure is not possible, radiation can provide palliation or extend progression-free survival in conjunction with systemic therapies. However, in many patients, the dose of radiation that can be safely administered is insufficient to achieve high rates of local tumor control and cure. In others, normal tissue injury may be a concern even at moderate doses. Ideally, in these settings, radiation would be combined with drugs that can enhance its tumoricidal effects (local or even abscopal) but without or only little added toxicity (Bristow et al. 2018; Baumann et al. 2016; Kirsch et al. 2018; Lin et al. 2013b).

1.1 Molecular Targeted Drugs

Over the past two decades, cancer therapy has been revolutionized by personalized (or precision) medicine, with prominent examples being the use of small molecule inhibitors against chronic myeloid leukemia driven by the BCR-ABL fusion protein or non-small cell lung cancer (NSCLC) cancers with oncogenic mutations in the epidermal growth factor receptor (EGFR) (Cohen et al. 2002; Lynch et al. 2004). Molecular targeted therapy can be defined as blocking a target that controls biological processes critical to the initiation and maintenance of cancer. Ideally, the target should be measurable in the clinic and measurement of the target should correlate with clinical benefit following administration of the targeted therapy (Sledge Jr. 2005). The arrival of targeted therapies has enabled oncologists to try to turn incurable cancers into chronic disease, or to at least achieve significant prolongations of progression-free survival (Chong and Janne 2013).

Importantly, many of the cellular pathways that promote tumor growth and survival may also play a role in response to treatment with ionizing radiation, suggesting that their pharmacological inhibition could cause tumor radiosensitization (Bristow et al. 2018). For example, while EGFR signaling may drive tumor growth in the small subset of NSCLC patients whose tumors harbor activating mutations in its tyrosine kinase domain, wild-type EGFR is expressed in the majority of lung and

	Precision Medicine	Precision *Radiation* Medicine
Endpoint	Response	Tumor control (kill all CSCs)
Intent	Chronic disease	Cure
Selectivity	Drug effective in a few	Radiosensitizer ideally effective in most tumors (similar to radiation effect)
Mechanism	Drug targets tumor dependence	• Radiosensitizing mechanism of action may be different from drug alone effect • Ideally not toxic by itself
Target	• Single drug target • Increasing use of drug combos	• Radiation has multiple effects • Sensitizer may need to hit >1 target or a central mechanism (DNA repair)
Biomarkers	Required for drug effect	• Understudied • To identify radioresistant tumors, or/and predict radiosensitization

Fig. 1.1 Comparison of precision medicine concepts in medical oncology vs radiation oncology aka precision radiation medicine. *CSCs* cancer stem cells

other cancers where it potentially can modulate responses to radiation (Baumann et al. 2007). However, targeting EGFR for radiosensitization has only been successful in an unselected population of patients with head and neck squamous cell carcinomas treated without chemotherapy and has failed in other clinical settings (Bonner et al. 2006; Bradley et al. 2020; Gillison et al. 2019; Ang et al. 2014).

Important differences in the utility of targeted drugs in mono-therapy versus their use as radiosensitizer likely exist and are summarized in Fig. 1.1. Traditionally, radiosensitizers have been regarded as effective in unselected patients, similar to the concept of combining radiation with chemotherapy. However, it appears increasingly possible that this "one-size-fits-all" approach is not viable in the clinic and that targeted agents only radiosensitize subsets of tumors, which would require predictive biomarkers to identify those patients who are most likely to benefit. Alternatively, biomarkers may be employed to identify radioresistant or radiosensitive strata of patients. The use of targeted agents with chemoradiation, which could be associated with increased toxicity, may only be justified in patients with radioresistant disease.

1.2 Predictive Biomarkers

Molecular targeted drugs can produce dramatic clinical responses in subsets of patients with disseminated cancer. The discovery of these agents has been concurrent with the characterization of the molecular genetic changes in an individual's tumor that can play a critical role in determining the clinical response to a particular

drug. Human cancers vary enormously in their somatic genetic alterations, and it is becoming widely accepted that these genetic differences, even in tumors with the same basic histological features, are the important determinants of response to these targeted drugs. The genomic characterization of human cancers that has been fueled by the successes of targeted drugs now also provides a basis for a more rational, biologically informed use of radiation therapy, with or without the addition of targeted radiosensitizers (Hall et al. 2018; Eke et al. 2016b; Kamran and Mouw 2018).

Analogous to the concept of precision medicine, "precision radiation medicine" may thus leverage genomic information derived from human cancers or preclinical tumor models to identify subsets that are sensitive to specific radiation/drug combinations, or radiation alone. Genomic biomarkers of radiosensitization may include oncogenic driver mutations, as increasingly found in for example lung cancers, or passenger mutations that do not affect tumor cell growth/survival in the absence of radiation exposure but that become important determinants of survival once cells suffer radiation damage. This remains a vastly understudied area, particularly in comparison with recent advances in matching drug-alone sensitivities to oncogenic driver mutations. Furthermore, as we are acquiring a deeper understanding of the hallmarks of cancer and how they may differ across individual tumors and patients, we will be in a better position to identify molecular targets for tumor radiosensitization (Fig. 1.2) (Willers et al. 2019). Importantly, many of the hallmarks of cancer are intimately linked to effects of radiation, examples being the impact of DNA repair alterations on radiosensitivity and the role of local immune escape on radiation response (Boss et al. 2014).

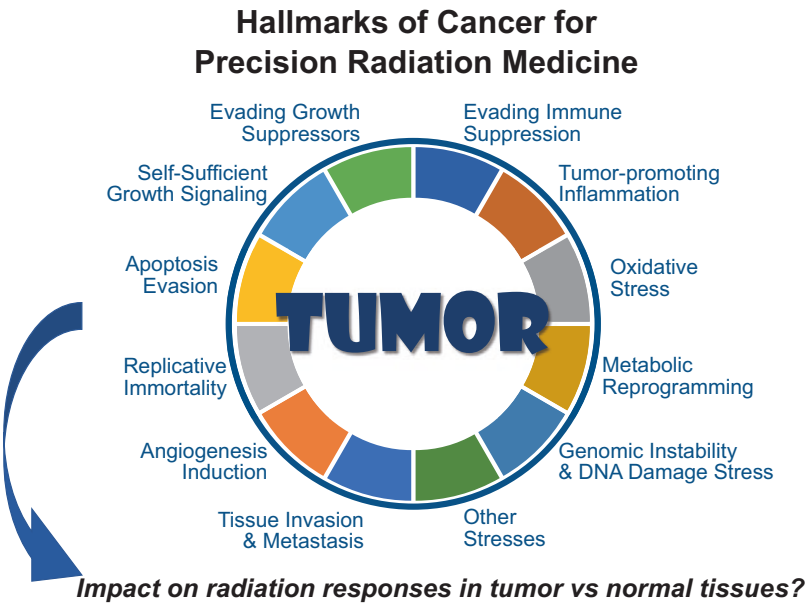

Fig. 1.2 How do the hallmarks of cancer impact tumor response to radiation treatment? (Redrawn from Willers et al. (2019))

1.3 Targeted Radiosensitizers and Immunotherapy

As we have firmly entered the era of immuno-oncology, what does this mean for the preclinical and clinical development of targeted radiosensitizers? In the future, immunotherapy rather than targeted radiosensitizers may be used to enhance tumor control and cures in many patients with solid tumors. However, it can be assumed that immunotherapy will not be of benefit in all cancer patients so that targeted radiosensitizers will retain their importance in at least subsets of patients. In addition, increasing evidence suggests that targeted radiosensitizers, particular DNA repair inhibitors, can modulate the immune response (Zhang et al. 2019; Konstantinopoulos et al. 2019; Vendetti et al. 2018). This opens up an exciting area for investigation into novel radiation/drug regimens. Lastly, immune checkpoint inhibitors may themselves have radiosensitizing properties (Azad et al. 2017; Deng et al. 2014; Crittenden et al. 2018).

Taken together, combining molecular targeted and immuno-modulating agents with radiation continues to show great promise both to radiosensitize tumors and to maximize protection of normal tissues. For many promising targeted agents and immune checkpoint inhibitors, one of their greatest impacts in oncology could ultimately rest in their combination with established treatment modalities such as radiation with/without chemotherapy to further cure and survival rates (Bristow et al. 2018).

2 Challenges for Targeted Radiosensitizers

Preclinical and clinical drug development with radiation therapy has been considered of critical importance to cancer research (Lawrence et al. 2013; Colevas et al. 2003; Harrington et al. 2011; Katz et al. 2009; Bristow et al. 2018). However, a large body of preclinical radiation/drug studies has not translated into an adequate number of successful radiation trials (Lawrence et al. 2013; Morris and Harari 2014). In fact, cetuximab remains to this date the only molecular targeted agent approved by the U.S. Food and Drug Administration—for use with radiation therapy in head and neck cancers (Bonner et al. 2006). A number of reasons likely exist, many of which have been discussed (Higgins et al. 2015; Lawrence et al. 2013; Lin et al. 2013b; Morris and Harari 2014; Coleman et al. 2016; Stone et al. 2016). Here, we wish to highlight what could be some of the most pressing challenges to the identification of successful radiation/drug combinations for the clinic.

2.1 Reproducibility of Preclinical Radiation Data

Preclinical evaluation of radiation effects is challenging due to the need to measure loss of replicative tumor cell potential, integrate concurrent chemotherapy which is the standard-of-care in many cancer types, and model the impact of the tumor

microenvironment (Morgan et al. 2014). Furthermore, radiosensitizing drug effects in clonogenic survival assays (CSA) are often small with dose enhancement factors much below 2. This stands in contrast to the effects of targeted drugs on in vitro measures of tumor response, i.e., a reduction in cell number/viability, which is often pronounced (Barretina et al. 2012; Garnett et al. 2012).

These challenges are compounded by shortcomings in the design and reporting of radiation/drug experiments according to a recent review of 125 publications by Stone and colleagues (Stone et al. 2016). The authors described a large number of instances in which experimental studies contained inadequate or unclear information (222 problems in 104 in vitro studies and 109 problems in 51 in vivo experiments). These issues could hamper efforts to replicate or compare the data and weaken the evidence for any subsequent clinical trials. Areas needing improvement include:

1. Authentication of cell lines and testing for pathogens such as mycoplasma
2. Sufficient information on drug source, storage, vehicle, preparation, concentrations, etc.
3. Description of radiation source, irradiation setup, dosimetry, and other factors, including traceability of output verification of X-ray tube to National Standards
4. Information on in vitro and in vivo drug administration schedules, including exact timing and relationship to irradiation, and the underlying rationale
5. Proper conduct of CSA
6. Information on number of independent biological repeats performed
7. Inclusion of full data set in supplement if representative data are shown
8. Appropriate statistical analysis of results in consultation with a statistician
9. Blinded counting of colonies and outcome assessments wherever possible
10. For mouse experiments, detailed descriptions that include tumor size at start of treatment, whether treatment was started when tumors reached a given size or at a given time after implantation. Tumors should be sufficiently large at the start of treatment to have biological properties of established tumors and to facilitate accurate measurement. Information on tumor transplantation procedure, site, method and frequency of measurement, etc.

The authors stressed that preclinical radiation/drug studies should meet standards of design, execution, and interpretation, and report necessary information to ensure high quality and reproducibility of studies. These improvements may provide a more robust basis for prioritizing drugs for clinical radiation therapy trials and for the design of such trials.

2.2 Modeling of Clinically Relevant Intertumoral Heterogeneity

Established cancer cell lines remain critically important for mechanistic studies of radiation/drug interactions, target validation, and in vivo confirmation as xenografts. Traditionally, radiation/drug combinations have been studied in limited numbers of

cell lines (Kleiman et al. 2013; Lin et al. 2014; Carmichael et al. 1987; Wang et al. 2001; Lally et al. 2007). However, any radiosensitizing effects in one or a few cell lines may not be representative of efficacy in an unselected larger number of genetically heterogeneous tumors, which may only be revealed when the agent under study has entered clinical trials. Historically, the choice of targeted radiosensitizers has conformed to a "one-size-fits-all" philosophy, but it is becoming increasingly possible that radiosensitizing effects are tumor genotype-dependent, which would require predictive biomarkers for appropriate patient selection (Lin et al. 2013a; Das et al. 2010; Liu et al. 2015; Willers and Hong 2015; Wang et al. 2018). Therefore, an appropriate number of human cancer-derived cell lines, for a given tumor type, may need to mirror the number of cell lines used in previous drug-alone screens, i.e., dozens per cancer type, given our emerging knowledge of the considerable genetic heterogeneity of tumors even within the same cancer type and histology (Cancer Genome Atlas Research Network 2012; Imielinski et al. 2012; Neve et al. 2006; Sos et al. 2009; Garnett et al. 2012; Barretina et al. 2012; Iorio et al. 2016). A larger number of cell lines would be needed to identify potential associations of radiosensitization with genomic alterations that have a low but still clinically relevant frequency of, for instance, 10–15% in the population.

The CSA has been considered the gold standard for assessing the cell-inactivating effects of radiation in vitro (Puck and Marcus 1956; Katz et al. 2008; Kahn et al. 2012). However, CSAs are not ideal for the kind of high-throughput screens that are needed to match diverse genomic tumor profiles with radiation/drug sensitivities owing to the frequently poor colony-forming ability of human cancer cell lines and the time it takes to conduct these assays. Short-term cell viability/survival assays, on the other hand, are historically not considered to provide appropriate surrogate endpoints of clonogenic survival (Lin et al. 2014; Brown and Wouters 1999; Brown and Wilson 2003). However, plate formats have been successfully tested and provide an opportunity for examining larger numbers of genomically characterized cancer cell lines and targeted drugs than have been historically pursued (Yard et al. 2016; Liu et al. 2015; Wang et al. 2014; Lin et al. 2014; Eke et al. 2016a). More work is required to validate these different approaches.

2.3 Integration of Experimental Approaches

Coleman and colleagues recently outlined a bench-to-bedside workflow to identify the most promising radiation/drug combinations for clinical testing (Coleman et al. 2016). This workflow involves an initial unbiased screening of cancer cell lines with radiation/drug combinations, followed by refinement and validation of "hits," after which tumor efficacy and treatment toxicity are assessed in appropriate animal models. An adapted preclinical workflow is shown in Fig. 1.3 and discussed below. Preclinical development of radiation/drug combos in such a manner is expected to be resource-intensive and time-consuming and requires integration of synergistic preclinical tumor models and capabilities of several institutions (e.g., NCI FOA PAR-16-111).

2.3.1 In Vitro Screening

As discussed above, initial testing of radiation/drug combinations may employ an appropriate number of authenticated cancer cell lines whose genomic and phenotypic characteristics are representative of the tumor type being studied. Treatment of one or a few cell lines with radiosensitizing agents likely produces biased results that will not translate into a more diverse tumor population. Drugs need to be given at multiple concentrations that are achievable in patients and have no or little toxicity by itself. Consideration should be given to pursuing more physiologic in vitro culturing conditions that better resemble in vivo tumor growth (such as use of 3D growth formats, extracellular matrix, physiologic oxygen concentrations, patient-derived cell line models and co-cultures). Initial investigations of immunotherapies or targeted drugs that interact with the tumor microenvironment have to be conducted in appropriate in vivo models such as genetically engineered mouse models (GEMM) (Castle et al. 2017) or perhaps in ex vivo systems (Jenkins et al. 2018).

2.3.2 In Vitro Validation

Screening results should be confirmed with CSA whenever possible. Additional assays may consider CSC-like cells that are relevant for radioresistance, for example, through the use of tumor spheres. In vitro validation may also include assessing the impact of concurrently administered chemotherapeutics on radiosensitizing drug effects although laboratory modeling of clinically relevant dosing and timing of chemotherapies is not trivial. The inclusion of patient-derived tumor models is recommended if the initial screen was done on established cancer cell lines. For other tasks, see Fig. 1.3. In general, this step narrows down the number of compounds that will undergo more expensive and time-consuming animal testing.

2.3.3 In Vivo Testing

Initial tumor models may be xenografts derived from genomically characterized cell lines used in the in vitro investigation or appropriate PDX models. Assessment of efficacy in murine models should consider treatments that are clinically relevant, including fractionated radiation and standard-of-care chemoradiation regimens. This approach provides initial assessments of drug efficacy, mechanistic insight as well as pharmacokinetic/pharmacodynamic measures. However, results from tumor growth delay assays may not always be consistent with the results of local control (TCD50) assays (Gurtner et al. 2011; Krause et al. 2006). TCD50 assays are performed much less commonly than growth delay assays because of the larger number of animals required and higher cost (Coleman et al. 2016). Nevertheless, before clinical trials with curative endpoints are initiated, TCD50 assays, which better reflect CSC inactivation, should be considered to reduce the chance of a negative trial. Additional animal models such as GEMMs harboring a natural tumor micro-

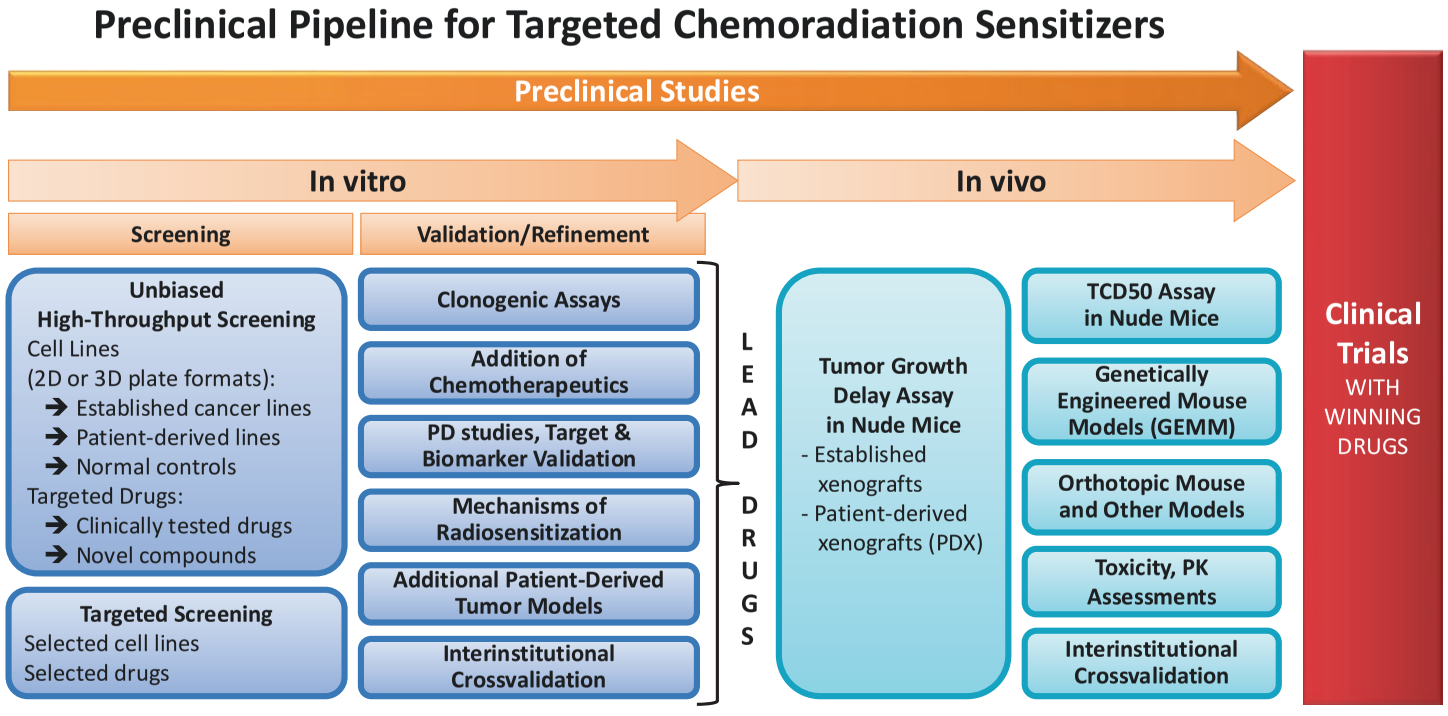

Fig. 1.3 Proposed preclinical pipeline for identifying "winning" targeted chemoradiosensitizers. (Inspired by Coleman et al. (2016))

environment and an intact immune system are also important components within the preclinical pipeline before bringing radiation/drug combos into the clinic (see Fig. 1.3).

The above considerations have served as motivation for this book, which provides a comprehensive review by experts in the field of key preclinical research components required to identify effective and safe (chemo-)radiosensitizing drugs. Readers are provided with a detailed and timely insight into the framework of targeted radiosensitizer research coupled with recent developments in immuno-oncology. Ultimately, this volume will support the identification of appropriately validated, and potentially biomarker-directed, targeted drug/radiation combinations that will have a higher likelihood than in the past to be incorporated into standard management of human cancers. These developments, coupled with the increasing technical power of radiation therapy to safely increase local control for many solid tumors, are expected to improve survival outcomes and cure rates for our patients.

3 Chapter Overview

Citrin and Camphausen (Chap. 2) provide a comprehensive review on the unique challenges that clinical translation and testing of targeted radiosensitizers present. These include how to best sequence agents and radiation, establishing biomarkers of efficacy, and integration into the current standard of care which includes cytotoxic chemotherapy in many settings. The authors conclude that an expanding knowledge of the underlying mechanisms of resistance and recurrence after radiation therapy coupled with the growing capacity to molecularly profile tumors provide great hope for future progress in this field. Abazeed and colleagues (Chap. 3) introduce the readers to preclinical studies of radiation responses and targeted sensitizers. They focus on the review our emerging knowledge of tumor and normal tissue genomics and their impact on the outcomes after radiation therapy (i.e., the "radiogenome"). They emphasize that given this knowledge population-based estimates of treatment effects increasingly cannot be justified. Critically, genomic tumor and patient features have considerable potential to serve as predictive biomarkers that can guide the clinical development of targeted radiosensitizers. Because targeted radiosensitizers or chemoradiosensitizers are expected to have particular utility in the treatment of radioresistant cancers, we summarize clinically relevant mechanisms of radiation resistance (Chap. 4). Of particular interest are tumor mutations in *KEAP1* as well as *KRAS*, which define an emerging area of need for intensification of radiation-based treatment regimens.

Starting a series of chapters on preclinical models for the study of targeted radiosensitizers, Lin and colleagues (Chap. 5) provide a comprehensive overview of preclinical strategies for testing of targeted radiosensitizers with a focus on clonogenic and non-clonogenic screening assays. They also review published guidelines and recommendations for the conduct of these studies. Three-dimensional (3D) cell cultures are well suited to model the extracellular matrix of tumors and provide more

physiological treatment responses than traditional 2D cell cultures, as reviewed by Cordes and colleagues (Chap. 6). In particular, radioresistance and the effects of radiosensitizing agents are effectively captured by 3D tumor models, which comprise an important level of investigation before moving drug testing into animals. Baumann and colleagues (Chap. 7) discuss the conduct of radiation and radiation/drug studies in different mouse models. While every model has advantages and disadvantages, the use of genomically defined heterotopic xenograft tumor models facilitates testing of clinically relevant radiation dose fractionation schedules and assessments of local tumor control. Kirsch and colleagues (Chap. 8) review the advantages of genetically engineered mouse models for the study of targeted radiosensitizers as well as radiation biology in general. These include preservation of the natural tumor microenvironment, ability to assess clinically relevant normal tissue injury, precise temporal and spatial control of genetic alterations that may affect radiation/drug responses, and lastly, the presence of an intact immune system.

Moving on to clinically promising therapeutic targets, Morgan and colleagues (Chap. 9) provide an in-depth review of clinically relevant small molecule inhibitors directed against kinases in the cellular DNA damage response (DDR). Of special interest is the recently recognized link of DDR targets to immuno-modulation which creates opportunities for novel radiation/drug regimens. The promise of targeting altered cellular metabolism, a hallmark of cancer, is comprehensively addressed by Schwarz, Allen, and colleagues (Chap. 10). Preclinical and clinical evidence supports the combined use of a number of metabolically targeted agents with radiation therapy, including those that affect nucleotide metabolism, glutaminolysis, oxidative stress, or iron metabolism. Hammond and colleagues (Chap. 11) provide an overview of tumor hypoxia, long known to limit the effectiveness of radiation therapy. Novel therapeutic approaches are emerging that include targeting oxidative metabolism in tumors. The authors emphasize the need for clinical development of hypoxia biomarkers without which patients most likely to benefit cannot be selected for hypoxia-targeted treatment strategies. Jain and Martin (Chap. 12) describe pioneering work on the effects that the altered tumor microenvironment has on cancer therapy outcomes including radiation. Novel approaches to inhibit tumor angiogenesis and desmoplasia can normalize the tumor microenvironment towards alleviating hypoxia and reversing radioresistance. The authors stress the need for appropriate preclinical models and imaging tools to identify optimal combinations of radiation with anti-angiogenic treatments, mechanotherapeutics, chemotherapy, and immune checkpoint inhibitors in the context of TME normalization. Lastly, Leeman and Schoenfeld (Chap. 13) provide an overview of the rapidly expanding field of radiation combined with immune checkpoint inhibitors, both with regard to enhancing local tumor control and eliciting abscopal effects. Because molecular targeted agents are being increasingly recognized as having immuno-modulatory effects, additional opportunities, as well as challenges, exist for multi-modality approaches that employ radiation and systemic combinations of chemotherapy, targeted agents, and/or checkpoint inhibitors.

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