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Review

REVIEW

The Future of Radiobiology

David G. Kirsch, Max Diehn, Aparna H. Kesarwala, Amit Maity, Meredith A. Morgan, Julie K. Schwarz, Robert Bristow, Sandra Demaria, Iris Eke, Robert J. Griffin, Daphne Haas-Kogan, Geoff S. Higgins, Alec C. Kimmelman, Randall J. Kimple, Isabelle M. Lombaert, Li Ma, Brian Marples, Frank Pajonk, Catherine C. Park, Dörthe Schaue, Eric J. Bernhard

Affiliations of authors: Department of Radiation Oncology and Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC (DGK); Department of Radiation Oncology, Stanford Cancer Institute, and Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA (MD); Radiation Oncology Branch (AHK, IE) and Radiation Research Program, Division of Cancer Treatment and Diagnosis (EJB), National Cancer Institute, National Institutes of Health, Bethesda, MD; Department of Radiation Oncology Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA (AM); Department of Radiation Oncology (MAM) and Department of Biologic and Materials Sciences, Biointerfaces Institute, School of Dentistry (IML), University of Michigan, Ann Arbor, MI; Department of Radiation Oncology, Washington University School of Medicine, St. Louis, MO (JKS); Department of Radiation Oncology, Princess Margaret Cancer Center, Toronto, ON, Canada (RB); Department of Radiation Oncology and Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, New York, NY (SD); Department of Radiation Oncology, University of Arkansas for Medical Sciences, Little Rock, AR (RJG); Department of Radiation Oncology, Harvard Medical School, Dana-Farber Cancer Institute, Brigham and Women's Hospital, Boston Children's Hospital, Boston, MA (DHK); Department of Oncology, University of Oxford, Oxford, Oxfordshire, UK (GSH); Perlmutter Cancer Center and Department of Radiation Oncology, New York University Langone Medical Center, New York, NY (ACK); Department of Human Oncology, University of Wisconsin School of Medicine and Public Health, Madison, WI (RJK); Department of Experimental Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX (LM); Department of Radiation Oncology, University of Miami, Miami, FL (BM); Division of Molecular and Cellular Oncology (DS), Department of Radiation Oncology (FP), David Geffen School of Medicine, University of California, Los Angeles, C A; Department of Radiation Oncology, Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA (CCP)

See the Notes section for the full list of authors and affiliations.
Correspondence to: David G. Kirsch, MD, PhD, Duke University Medical Center, DUMC Box 91006, Durham, NC 27708 (e-mail: david.kirsch@duke.edu).

Abstract

Innovation and progress in radiation oncology depend on discovery and insights realized through research in radiation biology. Radiobiology research has led to fundamental scientific insights, from the discovery of stem/progenitor cells to the definition of signal transduction pathways activated by ionizing radiation that are now recognized as integral to the DNA damage response (DDR). Radiobiological discoveries are guiding clinical trials that test radiation therapy combined with inhibitors of the DDR kinases DNA-dependent protein kinase (DNA-PK), ataxia telangiectasia mutated (ATM), ataxia telangiectasia related (ATR), and immune or cell cycle checkpoint inhibitors. To maintain scientific and clinical relevance, the field of radiation biology must overcome challenges in research workforce, training, and funding. The National Cancer Institute convened a workshop to discuss the role of radiobiology research and radiation biologists in the future scientific enterprise. Here, we review the discussions of current radiation oncology research approaches and areas of scientific focus considered important for rapid progress in radiation sciences and the continued contribution of radiobiology to radiation oncology and the broader biomedical research community.

Role of Radiation Biology Research in the Research Enterprise

Past research into the cellular response to ionizing radiation led to fundamental biological insights, such as demonstrating the existence of stem/progenitor cells (1) and identifying key components of the DNA damage response pathway (2). These and current advances in radiobiology impact clinical radiation oncology, improving cancer patients' outcomes (3). Basic insights from radiobiology can be applied to societally important topics

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such as carcinogenesis risk estimation from medical, occupational, or space travel radiation exposure (4) and the development of medical treatments for radiation injury (5). Despite the substantial impact of radiation biology in the past and its potential for future contributions, the field of radiation biology is facing challenges in research workforce, training, and funding, exacerbated by a clinical emphasis on technological rather than biologic science advances. Therefore, the National Cancer Institute (NCI) convened a workshop to discuss the role and future of radiation biology research and radiation biologists in the context of radiation oncology.

The radiobiology research workforce faces ongoing challenges of critical mass and identity that have been a topic of discussion for more than a decade (6). The number of principle investigators whose research focuses on radiation biology is limited, and researchers in radiation oncology departments often define themselves as cancer biologists or immunologists, rather than as radiation biologists. While this diversity of expertise is a strength, maintaining a research focus that is clinically relevant to radiation oncology is essential to improving patient care.

For radiobiology, as for other scientific disciplines, scientific reproducibility is an absolute requirement. To facilitate replication of radiation research, it is particularly important to standardize radiation dosimetry (7) and sufficiently describe experimental details of combining drugs and radiation (8). Rigorous application of basic radiobiological principles and techniques will enhance the reproducibility and scientific impact, but this requires maintaining a workforce with an advanced working knowledge of these basic principles.

Previous surveys and workshops have reported that the number of National Institutes of Health (NIH)–funded researchers within radiation oncology departments is small, which reflects a small and shrinking applicant pool (9). However, the success rate of radiobiology applications has not been substantially different from that of other oncology disciplines. To update these reports, we conducted a survey of FY2016 radiation-related federal awards using the NIH RePORTER search engine (https://projectreporter.nih.gov/reporter.cfm). There were 72 304 projects reported in this fiscal year (including multiple reporting of multiproject awards), 634 of which were retrieved in our search focused on ionizing radiation studies. Review of the abstracts was done to ensure that only awards directly exploring radiation-related topics were counted. Topics with relevance to radiation (eg, DNA repair, cancer stem cell studies) that did not mention studies with ionizing radiation were not included; thus the results are a conservative estimate. Two-hundred ninety-two awards were identified, funded through the various NIH institutes and centers, primarily the NCI, as well as through the US Food and Drug Administration and Veterans Administration under a variety of grant and contract mechanisms (Table 1; Supplementary Table 1, available online). The majority of these (n = 183) focused on radiobiology, with 56 awards focused on a clinical question and 15 awards whose scope included both.

A concern raised by Steinberg et al. in 2013 was that most radiation researchers with an NIH grant were full professors, while only 4.6% of the grants were career development awards, indicating a limited pipeline of early career investigators within the radiation sciences (9). Our RePORTER survey showed that this trend continued in 2016, with 11 (3.8%) K-type awards or K99/R00 career development awards identified. However, these data were somewhat mitigated by the finding that 27 (9.4%) awards were awarded to investigators who were identified as new or early-stage investigators. Nevertheless, it is evident that as established radiation researchers retire, investing in training junior investigators to perform radiation research and teaching will be essential to maintaining the field's vitality (10,11). To retain clinical radiobiology expertise, increased emphasis on training and retention of PhD- and MD/PhD-level radiobiologists by academic radiation oncology departments will be key, as well as supporting active research programs in these departments.

Table 1. Overview of radiation-related grants awarded in FY2016

Primary topics of radiation research awards	No. of awards	Training/ career development awards	No. of awards
Biology	183	Training T32	4
Clinical	56	Training R25	2
Clinical and biology	15	K-awards	9
Chemistry	5	K99/R00	2
Physics	5	–	–
Countermeasures	4	–	–
Epidemiology	4	–	–

Successful development of new physician scientists in radiation oncology will require opportunities for mentored research training extending beyond residency. Trainees making the transition to scientific independence should be encouraged to apply for K08, K99, and other career development awards. Academic departments must invest in junior scientists by providing both mentoring and sufficient support to develop a research program. For clinician scientists, protected time and adequate technical support are essential. In addition, clinician scientists can initially be embedded as mentored but independent researchers within a laboratory of an established scientist, thereby reducing practical laboratory management duties. Newly independent researchers in radiation research should apply to diverse funding sources, including NIH agencies, the Department of Defense, NASA, pharmaceutical companies, and various foundations (eg, American Cancer Society, Capcure, Lustgarten, etc.).

Given the challenges of maintaining a critical mass of investigators in the radiation research workforce, here we review current radiation research approaches and areas of scientific focus that should be considered for future investment.

Model Systems for Studying Radiobiology

Clonogenic survival is the gold standard assay for assessing radiation sensitivity in vitro as well as for testing the efficacy of agents that modify radiation survival. The advantage of this assay is that its colony formation end point integrates all forms of cell death and measures the reproductive capacity of individual cells, thus defining a cell's ability to replicate and form a tumor (12). While clonogenic assays are sensitive across a range of radiation doses, they are relatively low throughput and may not be suitable for all cells. Throughput is being addressed through development of medium/high-throughput-adapted colony formation assays (13). These screens have nominated targets for radiosensitization that have been validated for efficacy and selectivity and subsequently advanced to drug development programs (14).

To enable higher-throughput siRNA, CRISPR/Cas9, or small molecule library screens with radiation, investigators use multiwell plate formats with surrogates for clonogenic radiation

survival including radiation-induced foci (eg, γ–H2AX) (14,15), viability measured by Adenosine Triphosphate (ATP), or luciferase-based reporters (16,17). Clonogenic assays should still be done to validate the hits in these screens because they do not measure clonogenic survival.

For in vivo radiobiology studies, orthotopic (18) and subcutaneous Patient Derived Xenograft (PDX) models are rapidly being integrated as model systems in radiation research. Orthotopic models better approximate the tumor microenvironment of human tumors than do subcutaneous tumors, an important consideration for measuring radiation responses. However, orthotopic models introduce challenges for radiation delivery. The availability of small animal radiation micro-irradiators equipped with computed tomography (CT) imaging has made irradiation of orthotopic tumors feasible, but image-guided radiotherapy (IGRT) in mice can be limited by cost, throughput, and radiation toxicity when treating specific organs. Because PDX tumors are normally implanted into immune-deficient mice, this limits opportunities to study the immune system contribution to radiotherapy response, which has been shown to have an impact on radiocurability in syngeneic mouse models (19). The radiosensitivity of DNA-PK-deficient host mice is also a concern in radiobiological studies. Genetically engineered mouse models (GEMMs), while not fully recapitulating the genetic complexity of human tumor counterparts, generate autochthonous tumors within a native microenvironment in an immune competent animal, thus providing a critical model for dissecting mechanisms of tumor responses to radiation (20–23). GEMMs and GEMM-derived tumors (20–23) have been used for assessing experimental radiation sensitizers, including those targeting immune checkpoints (24).

The therapeutic potential of radiosensitizing agents is determined both by efficacy against and selectivity for tumor cells. Animal models of dose-limiting toxicities are more reliable than in vitro models for assessing normal tissue radiation toxicities, given the limitations of established cell lines in culture (eg, poor colony-forming efficiency, nonphysiologic in vitro growth conditions). Mouse models are routinely used for monitoring normal tissue toxicity such as those occurring in the oral mucosa and small intestine, which limit radiation dose in the clinic for head and neck and pancreatic cancers, respectively (25,26). Other animal models can be of high value in translational radiobiology, including swine and canine models of normal tissue injury (27,28) and companion animals with spontaneous tumors as advanced models for studying responses to radiation and combined modality treatments (29).

There is also great interest in using 3D models for radiobiological studies (eg, spheroids, organoids). These maintain some of the key physiologic and structural features of tumors (eg, cell-matrix interactions, hypoxia). They are less expensive than in vivo models, can be genetically modified, and can be employed for large-scale experiments (30). These models may better predict response than 2D cultures (30).

Molecular Targets and Radiosensitivity

A major goal of radiobiology is to achieve selective radiosensitization of cancer cells by modulating the molecular response to radiation injury. Ataxia-telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3 related (ATR) are key proteins in the DNA damage response pathways (31). Substrate analysis has identified more than 900 phosphorylation sites containing a consensus ATM or ATR phosphorylation motif in 700 proteins that are phosphorylated in response to ionizing radiation (32). These proteins regulate DNA repair, RNA post-translational modification, and cell morphology, suggesting opportunities to enhance tumor radiosensitivity by modulating ATM or ATR pathways.

ATM inhibitors have been shown to preferentially radiosensitize p53 mutant tumor cells in mouse xenografts when delivered via osmotic pump, thus offering some indication of selectivity (33,34,35). The orally available ATM inhibitor AZD0156, which has sub-nanomolar potency in cell-based assays with selectivity of greater than 1000-fold over other kinases such as ATR, shows synergy with DNA double-strand break-inducing agents in mouse xenograft models (36). A phase I clinical trial is currently testing AZD0156 alone or with other systemic drugs such as the PARP inhibitor olaparib in patients with advanced cancer (NCT02588105). There are no trials of ATM inhibitors with radiotherapy to date, and it will be important to determine whether this combination will have acceptable toxicities.

Two ATR inhibitors are currently in clinical trials. VE-822, also known as VX-970, radiosensitized pancreatic cancer cells as a single agent both in vitro and in xenograft models and synergistically sensitized lung cancer cells to cisplatin (37,38). Based on these preclinical data, a phase I trial is testing VX-970 with whole brain radiotherapy for patients with brain metastases from non–small cell lung cancer (NCT02589522). VX-970 is also being tested with radiation therapy and cisplatin for patients with human papillomavirus (HPV)–negative head and neck squamous cell carcinoma (NCT02567422). Another ATR inhibitor, AZD6738, is in a phase I trial as a single agent in combination with radiation therapy for refractory solid tumors (NCT02223923) (39,40).

The epithelial-to-mesenchymal transition (EMT), which generates cells with stem cell properties, is another target for radiosensitization. EMT can be modulated by extracellular factors, such as transforming growth factor-beta, tumor necrosis factor-alpha, and platelet-derived growth factor, or transcription factors, such as Twist, Snail, and zinc finger E-box-binding homeobox 1 (ZEB1) (41). Cancer stem-like cells preferentially activate the DNA damage response and repair pathways, thereby promoting radiation survival (42,43). A mechanistic link between EMT and radiation response signaling is that ZEB1 is phosphorylated and stabilized by ATM in response to DNA damage. Phosphorylated ZEB1 appears to promote DNA repair via USP7-mediated stabilization of checkpoint kinase 1, a process that can be reversed via microRNA (miR)-205 to enhance radiosensitivity (44,45). MiRs have yet to be approved by the US Food and Drug Administration for therapeutic use but are candidates for therapeutic interventions. Off-target effects may, however, complicate their clinical use, as was seen in a phase I trial of the liposomal miR-34 mimetic MRX34. MRX34 was used to downregulate oncogene expression (NCT01829971), but the trial was terminated due to immune-related adverse events.

The phosphatidylinositol-4,5-bisphosphonate 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway is another example of a pathway that has been studied extensively for its impact on radiosensitivity through a number of mechanisms (46,47). A critical question in the translation of molecular therapeutics to clinical trials with radiation is how to best test molecularly targeted agents. Stratification of patients to therapies by tumor molecular features is routinely utilized for systemic therapy trials, and this approach should be better integrated into clinical trials of radiation therapy. Developing and applying biomarkers to

radiotherapy trials, particularly when testing radiation response modulators, would be an important advance.

Metabolism as a Therapeutic Target

Otto Warburg observed in 1924 that tumors consumed large quantities of glucose while secreting high levels of lactate, irrespective of tissue oxygen concentration, a phenomenon now known as the "Warburg effect." This "aerobic glycolysis" has since been shown to benefit tumor growth by providing intermediates needed to maintain high rates of cellular division (48). In addition, tumor-specific genetic alterations drive metabolic phenotypes. For example, *RAS*-driven cancers use alternate carbon sources (both extracellular and intracellular) as fuel for the Tricarboxylic Acid (TCA) cycle (49). *MYC*-driven cancers engage in glutaminolysis, while other cancers may not require glutamine for growth (50). Thus, it is important to consider metabolic therapies in the appropriate genetic context.

Within tumor cells, high rates of glucose metabolism are needed to support intracellular redox balance. The connection between glucose metabolism and redox stress is relevant for radiation therapy. The consumption of glucose via the pentose phosphate pathway generates ribose, which can be used to generate nucleotides for DNA replication and repair; however, an important byproduct of this reaction is NADPH, which can be used by tumor cells as a source of reducing equivalents. Radiation increases intracellular redox stress. Therefore, interfering with metabolic pathways that support intracellular redox balance may enhance the efficacy of radiation (51,52). Tumors can use alternative fuel sources and rely on additional pathways to maintain reducing equivalents, which may also be attractive therapeutic targets.

Additional study is needed to characterize normal vs tumor metabolism and the impact of this difference on radiosensitivity. Also, understanding radiation effects on tumor metabolic phenotypes is needed in order to devise rational combinations of radiation and drugs that target tumor metabolism. Given the diversity in tumor metabolic phenotypes and the influence of specific gene mutations, it is critical to perform these studies in a defined genetic context. For these studies, it will also be important to understand the metabolic impact of the interaction between tumor and stroma using in vivo model systems and to harness the power of imaging to track treatment-associated changes in tumor metabolism after radiation, drug, or combined treatment.

Cancer Stem Cells

Cancer stem cells (CSCs) were initially described in early 2000 as a cell population capable of tumor regeneration (53,54). CSCs differ from their progeny in their metabolic state, which can convert easily between aerobic glycolysis and oxidative phosphorylation depending on the local microenvironment (55,56). CSCs increase the expression of enzymes that regulate intracellular redox metabolism (57,58,59) and activation of DNA repair mechanisms (42), and as such may represent a unique cell population for radiation therapy.

It has also been shown that Glioblastoma Multiforme (GBM) CSCs can migrate within the brain to the subventricular zone (SVZ) and thereby escape regions of high radiation dose (60,61). Environmental stress such as radiotherapy, chemotherapy, hypoxia, and low pH can increase CSC subpopulations and influence radiation sensitivity, and it has been proposed that some normal and non-neoplastic cells can convert spontaneously to a stem-like state (62).

In the future, methods and criteria for the identification of radiobiologically important CSCs should be developed and standardized. Further study is needed to understand CSC migration and determine whether CSC safe harbors like the SVZ need to be included in radiotherapy treatment fields. It may be possible to target the invasive phenotype of CSCs for therapy. Finally, further study is needed to understand how phenotypic plasticity influences radiotherapy outcomes. Drugs that limit phenotypic plasticity in tumors should be incorporated into radiotherapy clinical trials.

Radiation Toxicity to Normal Tissues and Stem Cells

Following ionizing radiation, cell death is initiated when cells with chromosomal aberrations attempt to divide. Thus, the timing of radiation toxicity in normal tissues often correlates with the cell cycle rate within that organ (63). Acute radiation toxicity occurs quickly in highly proliferative tissues, while slower-cycling organs often show late radiation responses. An exception is the salivary gland, which has a slow turnover rate yet responds acutely to radiation. Radiation first affects the plasma membrane of secretory cells, resulting in the disruption of stimulated water secretion and loss of organ function before any parenchymal cell death. Therefore, acute toxicity can derive from multiple factors in addition to cell loss. Intermediate to late side effects of radiation toxicity may include inflammation, fibrosis, loss of endothelial function (64), and potential neuronal dysregulation (65). When a given radiation dose exceeds an organ's tolerance, reduced and progressive loss of organ function can occur and persist for the patient's lifetime. Organ recovery after radiation depends on the number of surviving and functional stem/progenitor cells (66), which continuously replenish differentiated cell types within the organ. Multiple epithelial stem/progenitor cells are distributed along the organ to locally balance homeostasis by providing new cells, but also by cross-communicating with cells in the surrounding niche (67), such as stromal, endothelial, and neuronal-derived cells. Radiation not only impacts epithelial stem/progenitors, but also their niche's internal communication network to induce survival in neighboring cells and promote repair.

Efforts to reduce radiation toxicity are primarily focused on prevention and mitigation strategies. Preventative radioprotectors have tremendous potential, but clinical adoption has been hampered by concern over potential radioprotection of tumor and/or side effects. Mitigators delivered after radiation, which suppress cell death and/or enhance cell proliferation of surviving stem/progenitor cells, may prevent loss of organ function by impacting one or more cell types. To minimize organ dysfunction, an optimal radiation delivery technique to limit normal tissues to radiation should be combined with novel radioprotectors and local delivery of organ-specific radiation mitigators (68,69). The timing of applying these factors (ie, simultaneous or sequential) will be critically important as delivering antioxidants, prosurvival, pro-proliferation, and/or anti-inflammatory agents at the wrong time points may reduce their effects and potentially even enhance toxicity. Using sophisticated radiation delivery methods to avoid irradiating the most sensitive areas within an organ may also reduce toxicity, as has been described for salivary glands (70). Predictive models can be used to assist in minimizing the dose to critical areas, where potential clusters

of crucial epithelial stem cells and essential surrounding niches reside. Further study of regenerative therapies in the context of radiobiology, including cell transplantation, gene transfer, and tissue engineering, will provide novel approaches to repair radiation-damaged organs. Combining spatio-temporal radiation planning with protective and mitigating strategies may be the optimal approach.

Combining Radiotherapy With Immunotherapy

Radiation therapy has historically been a means of improving local control. However, mounting preclinical and clinical evidence indicates that radiation therapy can augment a systemic immune response directed against tumor cells when combined with immunomodulatory drugs (71). In some preclinical studies, mice bearing two synchronous tumors were used in order to test the ability of radiation delivered to one tumor to induce an out-of-field response in the other tumor (abscopal effect). Such responses reflect the development of robust antitumor T cells and have been achieved when radiation is combined with immunotherapies, such as anti-CTLA4 or anti-PD-1 antibodies, that are otherwise ineffective in these tumor models (72–74).

Several case reports, retrospective series, and a recent prospective trial support the clinical relevance of the observations made in preclinical models. In melanoma, Postow et al. described marked regression of nonirradiated metastases in a patient who had progressive disease on the anti-CTLA4 antibody ipilimumab and was subsequently treated palliatively with radiation to a paraspinal mass (75). Since the approval of ipilimumab for melanoma, several retrospective analyses have reported abscopal responses in patients treated with radiation during/after immunotherapy (76,77). The results of a phase I clinical trial that combined ipilimumab with hypofractionated radiation in patients with metastatic melanoma were recently reported, showing a response rate of 18% in unirradiated lesions (74). Responses to combination therapy have also been reported in tumors that did not initially show a clinical response to ipilimumab alone (78), suggesting that radiation could reposition immunotherapies for cancers that are not optimal candidates for these treatments. Importantly, a phase I–II trial of combination radiotherapy with granulocyte-macrophage colony-stimulating factor produced objective abscopal responses in more than 25% of patients with metastatic solid tumors, establishing radiotherapy as a potential in situ antitumor vaccine (79).

The mechanisms by which radiotherapy promotes a tumor-specific immune response are multifactorial and have been reviewed elsewhere (80–82). Radiation may act as a personalized vaccine by causing the release of danger signals and tumor-specific antigens, which are subsequently presented by dendritic cells to elicit a T-cell-mediated immune response (83–85). Because radiation can activate immune-inhibitory pathways as well as immune-stimulatory ones, the net effect of these opposing forces may ultimately determine whether radiation will drive a beneficial immune response in a specific patient (86). Thus, identification and targeting of the immunosuppressive pathways that are induced or exacerbated by radiation will be critical (87).

The notion that a healthy immune system contributes to successful radiotherapy has been around for decades (19), as has the immense potential that lies in combining radiation with immunotherapy (88). These concepts can now be revisited with newer immune targeting strategies. For example, radiation could convert an immune-privileged tumor into one that is T-cell-inflamed, which immune checkpoint inhibitors alone seem unable to do. Clinical testing and further mechanistic research will provide answers to outstanding questions to establish the role of radiation in immuno-oncology (89). Studies should be designed to assure that the contribution of radiation can be assessed, keeping in mind that immunotherapy can show delayed tumor responses. The radiation dose and fractionation and sequencing with immunotherapy that provide the optimal effect remain to be established, but recent mechanistic studies may provide a rationale for dose selection (90,91). Whether the site that is irradiated matters for radiation to potentiate an immune response is also unknown, and the main antigenic targets of radiation-induced immune responses are unclear. Most importantly, the identification of useful biomarkers that predict which patients will eventually have a clinical response is an important area of investigation that may guide patient selection and early evaluation of treatment response. In this regard, expression of Trex1, an upstream negative regulator of radiation-induced antitumor immunity, was recently shown to be a potential biomarker for determining the optimal radiation dose and fractionation to promote immune activation (91). Perhaps one of the great challenges and opportunities for the radiation oncology field will be to undertake big data studies that integrate sophisticated imaging technology and personalized radiation treatment planning with complex, multidimensional immune monitoring data and patient outcome (92,93).

Hypoxia, Tumor Vasculature, and Radiotherapy

The solid tumor microenvironment (TME) is often characterized by poor vasculature and inadequate blood supply (94). As a consequence, tumors may contain regions exhibiting increased interstitial fluid pressure, hypoxia, excess lactate accumulation, lowered pH, and a lack of nutrients. This hostile microenvironment may select for cells that are particularly resistant to killing, and may also contribute to phenotypic diversity within the tumor. However, reliable technologies to properly measure or predict outcomes based on average pH, interstitial fluid pressure, or oxygenation remain elusive.

Hypoxia is associated with poor prognosis in many cancers, irrespective of treatment (94–96). Hypoxic cells are more resistant to radiotherapy and some chemotherapies because O_2 participates in chemical reactions that enhance initial DNA damage, so without O_2 there is less DNA damage (96). Additionally, hypoxia influences biological signaling pathways, including the activation of the transcription factor hypoxia-inducible factor 1 (HIF-1), which promotes changes in cellular metabolism and other pathways that affect radiation response (20–23). Hypoxia-induced biological changes have been shown to promote the generation and maintenance of cancer stem cells in discrete regions of the tumor (43), which, as discussed above, may be more resistant to DNA damage. Hypoxia may also create an immunosuppressive tumor microenvironment that promotes evasion of immune surveillance. Lastly, hypoxia can promote malignant progression by increasing genomic instability and metastatic potential (97).

Specifically relating to hypoxia and radiation therapy, hypoxic cell radiosensitizers (nitroimidazoles) are reduced selectively in hypoxic cells to form covalent cross-links and also act to fix radiation damage. Some nitroimidazoles have shown modest benefit in clinical trials (98). Bioreductive prodrugs are

also being studied as hypoxic cytotoxins; some of these are now in clinical trials (99). The alternative approaches of reducing tumor hypoxia using hyperbaric oxygen during radiotherapy or administering a gas mixture containing higher levels of O_2 in combination with vasodilators (ARCON) have also shown modest improvements in local control (100).

An approach currently under investigation is the reduction of hypoxia by decreasing tumor O_2 consumption. Based on mathematical modeling, Secomb et al. predicted that even a 30% decrease in O_2 consumption would decrease the hypoxic fraction from 37% to 11% (101). Studies have shown metformin reducing O_2 consumption and tumor hypoxia, which may enhance radiation sensitivity selectively in hypoxic tumors (98,102).

In addition to directly targeting hypoxia within tumors, another therapeutic approach is to target the aberrant vasculature within the TME. Many strategies have been tried, ranging from starving tumors by collapsing their blood supply with vascular-disrupting agents to reducing hypoxia via vascular normalization (103). More recent work has shown the contribution of myeloid cells to vasculogenesis and metastasis after therapy, and the ability to block their recruitment to tumors by disrupting the CXCR4/CXCL12 interaction (104,105). These novel approaches to targeting or normalizing tumor vasculature, and to preventing revascularization after therapy, may improve treatment outcomes. Moreover, characterizing the hypoxia status of the tumor may help identify tumors that will recur or metastasize after primary therapy (106,107). Lastly, the field of nanomedicine is well positioned to develop multifunctional agents that preferentially act in or around the tumor microvasculature. These platforms have the potential to facilitate both imaging for and therapy with radiation (108–110).

There is growing evidence from a number of groups that radiation itself may lead to vascular dysfunction in certain settings, for example, when using doses on the order of 10 Gy or more per fraction (111–113). These are the types of doses used in stereotactic body radiotherapy (SBRT). As SBRT is increasingly used in the clinic, it is important in the future to understand how much of its efficacy is due to direct tumor cell killing vs indirect killing via vascular ablation. Experiments using dual recombinase technology in a genetically engineered mouse model of soft tissue sarcoma indicate that the killing of blood vessels contributes to growth delay, but not local control following SBRT (20–23). Further experiments in other model systems are needed to determine if these conclusions extend to other tumor types. In addition, a better understanding of how much the presence of hypoxia within tumors (and what specific type or signature of hypoxia) contributes to local failures after SBRT and whether adjuvant therapies such as vascular-targeted nanoparticles or high-frequency ultrasound ablation (HiFU) tumor debulking can increase local control following SBRT may expand the indications of SBRT to more tumor types and anatomical sites.

Extracellular Matrix and Physical-Mechanical Properties of the Tumor Microenvironment and Radiotherapy

The extracellular matrix (ECM) is another key factor in the tumor microenvironment affecting cancer cell survival after radiation. ECM comprises the structural, cellular, and molecular stromal components including the basal lamina or basement membrane that physically separate the epithelial compartment from the stroma (114). The integrin family of receptors, necessary for cell adhesion to the basement membrane, has largely defined adhesion-mediated signaling between the epithelial and stromal compartments. Integrin-mediated cell–ECM interactions can enhance radiation and chemoresistance of several tumor types by activating prosurvival signaling (115–118). Furthermore, adhesion of tumor cells to ECM proteins modulates the efficacy of molecularly targeted therapy (119,120). Therefore, a better understanding of the biochemical signaling pathways from the ECM should lead to new targets for sensitizing tumors to radiation and other therapies. In addition, investigating the mechanical signals that impact cell survival is now possible due to advances in the field of biophysical mechanics in cell biology (121).

Integrins induce biochemical signaling from direct binding of ECM ligands via multiple possible combinations of integrin heterodimers and ECM molecules. Further complexity lies in the specific physical and mechanical properties of individual ECM molecules, which can enhance integrin signaling by facilitating increased ligand-receptor engagement (122). The biophysical properties of ECM and tissues play a critical role in cellular function and signaling (123). Tumors are inherently stiffer than their normal tissue counterparts, and stiffness itself has been shown to enhance tumor progression (124). Indeed, multiple types of receptors and signaling that respond to biophysical changes in TME are now evident (114), including the YAP/TAZ cotranscription factors downstream of the HIPPO pathway, which have oncogenic properties (125). Signaling via HIPPO-YAP/TAZ ties developmental pathways regulating proliferation and organ size with cancer pathophysiology. Tumor stiffness regulates response to growth factor–mediated signaling via YAP/TAZ, demonstrating the broad relevance of this signaling network related to biophysical properties (119). Advanced 3D models reproducing physiologically relevant microenvironments and capable of tuning elastic modulus and microfabricating the size and shape of culture platforms with "stretch" capability will improve our understanding of the molecular and mechanical mechanisms underlying cancer and cancer cell survival mechanisms under physiologic conditions and enhance the testing of innovative treatment strategies to combine with radiotherapy.

Predictive Biomarkers for Radiotherapy

Predictive biomarkers that allow rational selection of treatments for individual patients are playing a central role in the precision cancer medicine revolution (126). While biomarkers have transformed approaches for systemic cancer treatment, few predictive biomarkers are currently available for radiotherapy in the clinic (127,128). In many disease sites, there is considerable heterogeneity in outcomes, both with regard to tumor control and treatment toxicity. Thus, biomarkers that predict tumor control probability and normal tissue complication probability would advance the care of individual patients (129). Although few biology-informed parameters currently influence radiation therapy decisions in the clinic, a number of areas of active investigation promise to deliver novel predictive biomarkers in the near future (Table 2).

One promising avenue of research for biomarkers predictive of radiation response relates to the altered function of genes involved in DNA repair. Ionizing radiation kills cells through direct or indirect induction of DNA damage, with DNA double-strand breaks (DSB) being the most important lesion (130).

Table 2. Future of predictive tumor biomarkers in curative radiation oncology*

Biological parameter	Examples of candidate biomarkers	Association with radioresistance or radiosensitivity	Potential intervention(s)	Current clinical status
Number of clonogenic tumor cells or CSCs	Cell surface markers such as CD44	Higher baseline number of clonogenic cells or CSCs correlates with radioresistance	Higher radiation dose or radiosensitizer for high CSC content	Tumor volume is a surrogate for CSC number and influences RT dosing in clinical practice
Accelerated tumor cell repopulation	EGFR expression	Accelerated repopulation of clonogenic tumor cells during RT causes radioresistance	Shortening of overall treatment time limits number of clonogenic cells that need to be sterilized by RT	HNSCC histology used as surrogate in clinical practice to guide accelerated fractionation schemes
Tumor sensitivity to RT fraction size	No candidate markers currently exist to predict α/β of individual tumors	Some tumor types are associated with high sensitivity to RT fraction size (low α/β of < 10 Gy)	Hypofractionation (>2 Gy daily fraction size)	Breast or prostate histology used as surrogate in clinical practice to guide hypo-fractionation schedules
Tumor hypoxia	PET/MRI-based imaging markers Gene expression signatures	Tumor hypoxia reduces radiation damage to DNA, thereby increasing radioresistance	Combination of RT with hypoxic radio-sensitizer or dose increase to hypoxic tumor regions	Not yet used in clinical practice
HPV status	HPV16 DNA or p16 expression	HPV infection causes radiosensitivity, likely through interfering with DNA repair	Treatment de-intensification	De-intensified treatment to reduce toxicity in HPV+ HNSCC in clinical trials
Intrinsic radiosensitivity	DSB repair gene mutations, gene expression signatures, repair foci (eg, γ-H2AX), ctDNA response	Variation in ability of tumor cells to cope with radiation damage may cause radiosensitivity or radioresistance	Treatment de-intensification or intensification, respectively	Not yet used in clinical practice
Tumor genotype	Mutations in oncogenes such as KRAS, BRAF, EGFR, etc.	Tumor mutation status may correlate with radiosensitivity or radioresistance	Treatment de-intensification or intensification, respectively	Not yet used in clinical practice

*CSC = cancer stem cells; ctDNA = circulating tumor DNA; DSB = double-strand break; HNSCC = head and neck squamous cell carcinoma; HPV = human papilloma virus; RT = radiation therapy.

Cancers with defective DSB repair are, therefore, expected to display increased sensitivity to radiation. Markers of DSB repair defects, for example, mutations in or altered gene expression of DNA repair genes, might therefore be ideal predictive biomarkers (131–134). Unfortunately, data convincingly linking mutations in DSB repair genes such as BRCA1 to increased tumor radiosensitivity in the clinic have not yet materialized (135). Furthermore, recurrent mutations in individual genes are rare, and DNA damage response and repair networks are complex (136,137). Therefore, comprehensive studies examining the association of DSB repair pathway alterations and clinical radiosensitivity are an important area of future investigation. It is also possible that tumors with such alterations may be particularly sensitive to combined treatment with radiotherapy and DNA repair inhibitors, such as those targeting PARP1, ATR, or CHK1 (138).

Other molecular characteristics of tumors could serve as potential predictive biomarkers for radiation therapy. For example, numerous studies have shown that patients with HPV-positive head and neck squamous cell carcinomas have relatively good outcomes after treatment with radiation therapy alone or combined with chemotherapy, corroborating preclinical work demonstrating key roles for HPV oncogenes in modulating radiation sensitivity (139,140). Indeed, therapy de-escalation studies are already underway to test if less intense treatment can achieve similarly good outcomes with fewer side effects in this patient population (NCT01084083, NCT01525927, NCT01716195, NCT01663259, NCT01302834, NCT01898494) (141). Mutations in cancer driver genes may also affect tumor radioresistance, but have been incompletely explored. Also, gene expression signatures that predict radioresistance/radiosensitivity warrant further exploration (142).

In addition to genomics-based biomarkers, the predictive power of assays that measure classic aspects of radiobiology also warrants further exploration. For example, functional assays of DSB, such as γ H2AX staining performed on biopsies before and after in vivo or ex vivo irradiation, could potentially serve as a direct indicator of patient-specific radiosensitivity (143,144). Current challenges of using such assays include the need for representative tumor biopsies and the use of fresh tissue, and more research is needed to develop techniques that can be more easily adopted in the clinic. Several groups are currently developing cell-penetrating anti-γ–H2AX tracers with the ultimate aim of using them as PET-based imaging tools to assess differences in DSB repair in radiotherapy patients (145). Furthermore, it would be useful to identify molecular correlates

of α/β ratios, which describe cell killing for tumor and normal tissues that could be employed to predict dose fractionation dependencies in radiotherapy (129). The use of increased fraction sizes in tumors with low α/β ratios, such as breast and prostate cancers, has already advanced into the clinic. However, α/β ratios currently cannot be measured for individual patients. If this were possible, fractionation regimens could potentially be individualized to maximize tumor cell killing while minimizing normal tissue toxicity.

Identification of predictive biomarkers for radiation therapy has been hampered by the fact that, except for prostate cancer studies (146), large multi-omics profiling efforts such as The Cancer Genome Atlas and the International Cancer Genome Consortium have by and large not included specimens from patients treated with radiation therapy. One technical hurdle for performing such studies is that only small amounts of tumor tissue are often available for radiotherapy patients. An attractive approach for overcoming this challenge is the use of liquid biopsies, including analysis of circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs) (147,148). An important advantage of liquid biopsy approaches is the ability to noninvasively and repeatedly sample molecular aspects of tumors. Liquid biopsies have a number of potential applications, including 1) noninvasive genotyping of tumors being treated with radiation therapy for which no or minimal tissue is available; 2) quantitation of tumoral heterogeneity as a biomarker; 3) early response assessment during radiation therapy to allow escalation or de-escalation of treatment; and 4) identification of radiographically occult residual disease after completion of radiation therapy to identify patients at high risk for recurrence. For example, presence of residual circulating tumor-derived EBV DNA in patients with nasopharyngeal cancer after treatment with radiation therapy predicts outcome (149). Modification of adjuvant therapy based on post-treatment EBV DNA levels is currently being explored in a prospective trial (NCT02135042). Based on recent advances in ctDNA detection technologies that focus on somatic mutations instead of viral DNA, similar approaches could likely be extended to most other cancers (150).

Identification of biomarkers that can predict outcomes prior to initiating radiation therapy will be an important advance. However, no single biomarker approach will likely achieve this goal; therefore, combination biomarkers that include measuring multiple analytes should be explored. Two such developing strategies include genomic-adjusted radiation dose (GARD) (151) and Post-Operative Radiation Therapy Outcomes Score (PORTOS) (152). Such gene expression–based platforms may be able to personalize radiotherapy tumor dose or the need for postoperative radiation. Biomarker studies will need to be performed rigorously and should adhere to "best practices" guidelines such as STARD and REMARK (153,154). Any predictive biomarker that is discovered will need to be validated in independent cohorts. Finally, the most rapid progress in this field will likely be through collaborative efforts and multi-institutional teams. If successful, predictive biomarkers hold the promise to transform both our understanding of radiobiology and the clinical management of patients treated with radiation therapy.

Conclusions

The field of radiobiology has made and continues to make critical contributions to science. To continue this work, challenges to the training and retention of the radiation research workforce and limitations in radiobiology research funding must be overcome. The potential for major advances in radiation research remains high because of the availability of new model systems, genome editing tools, and technology for genome-wide analyses. Decades of research investigating mechanisms of DNA damage response to ionizing radiation are now bearing fruit, with clinical trials combining radiation therapy with radiosensitizers that target these pathways. Continued investment in understanding mechanisms of tumor and normal tissue response to radiation can lead to a new generation of clinical trials to improve the therapeutic ratio of radiotherapy. Already, preclinical studies combining radiation with immunotherapy have led to a large number of clinical trials, which could transform radiation therapy from a local treatment to a treatment employed to achieve systemic tumor elimination. To have maximum impact for individual patients, predictive biomarkers should be identified that enable the rational selection of treatments to combine with radiation therapy. Further research into the radiobiology of tumor metabolism, cancer stem cells, and the tumor microenvironment has the potential to translate current knowledge and future gains to the clinic. Future investment by academic radiation oncology departments, professional societies, the NCI, and other biomedical research funding groups will ensure that the radiation research enterprise remains vibrant as a key contributor to increasing basic science knowledge and improving the outcomes of cancer patients.

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Authors: David G. Kirsch, Max Diehn, Aparna H. Kesarwala, Amit Maity, Meredith A. Morgan, Julie K. Schwarz, Robert Bristow, Sandra Demaria, Iris Eke, Robert J. Griffin, Daphne Haas-Kogan, Geoff S. Higgins, Alec C. Kimmelman, Randall J. Kimple, Isabelle M. Lombaert, Li Ma, Brian Marples, Frank Pajonk, Catherine C. Park, Dörthe Schaue, Phuoc T. Tran, Henning Willers, Brad G. Wouters, Eric J. Bernhard

Affiliations of authors: Department of Radiation Oncology and Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC (DGK); Department of Radiation Oncology, Stanford Cancer Institute, and Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA (MD); Radiation Oncology Branch (AHK, IE) and Radiation Research Program, Division of Cancer Treatment and Diagnosis (EJB), National Cancer Institute, National Institutes of Health, Bethesda, MD; Department of Radiation Oncology Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA (AM); Department of Radiation Oncology (MAM) and Department of Biologic and Materials Sciences, Biointerfaces Institute, School of Dentistry (IML), University of Michigan, Ann Arbor, MI;

Department of Radiation Oncology, Washington University School of Medicine, St. Louis, MO (JKS); Department of Radiation Oncology (RB), Princess Margaret Cancer Center (BGW), Toronto, ON, Canada; Department of Radiation Oncology and Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, New York, NY (SD); Department of Radiation Oncology, University of Arkansas for Medical Sciences, Little Rock, AR (RJG); Department of Radiation Oncology, Harvard Medical School, Dana-Farber Cancer Institute, Brigham and Women's Hospital, Boston Children's Hospital, Boston, MA (DHK); Department of Oncology, University of Oxford, Oxford, Oxfordshire, UK (GSH); Perlmutter Cancer Center and Department of Radiation Oncology, New York University Langone Medical Center, New York, NY (ACK); Department of Human Oncology, University of Wisconsin School of Medicine and Public Health, Madison, WI (RJK); Department of Experimental Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX (LM); Department of Radiation Oncology, University of Miami, Miami, FL (BM); Division of Molecular and Cellular Oncology (DS), Department of Radiation Oncology (FP), David Geffen School of Medicine, University of California, Los Angeles, CA; Department of Radiation Oncology, Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA (CCP); Department of Radiation Oncology and Molecular Radiation Sciences, Oncology and Urology, Johns Hopkins University School of Medicine, Baltimore, MD (PTT); Department of Radiation Oncology, Massachusetts General Hospital and Harvard Medical School, Boston, MA (HW); Departments of Radiation Oncology and Medical Biophysics, University Health Network, Toronto, ON, Canada (BGW).

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References

1. Till JE, Mc CE. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat Res*. 1961;14:213–222.
2. Kastan MB, Zhan Q, el-Deiry WS, et al. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. *Cell*. 1992;71(4):587–597.
3. Moding EJ, Kastan MB, Kirsch DG. Strategies for optimizing the response of cancer and normal tissues to radiation. *Nat Rev Drug Discov*. 2013;12(7):526–542.
4. Durante M, Cucinotta FA. Heavy ion carcinogenesis and human space exploration. *Nat Rev Cancer*. 2008;8(6):465–472.
5. Williams JP, Brown SL, Georges GE, et al. Animal models for medical countermeasures to radiation exposure. *Radiat Res*. 2010;173(4):557–578.
6. Coleman CN, Stone HB, Alexander GA, et al. Education and training for radiation scientists: Radiation research program and American Society of Therapeutic Radiology and Oncology Workshop, Bethesda, Maryland, May 12-14, 2003. *Radiat Res*. 2003;160(6):729–737.
7. Desrosiers M, DeWerd L, Deye J, et al. The importance of dosimetry standardization in radiobiology. *J Res Natl Inst Stand Technol*. 2013;118:403–418.
8. Stone HB, Bernhard EJ, Coleman CN, et al. Preclinical data on efficacy of 10 drug-radiation combinations: Evaluations, concerns, and recommendations. *Transl Oncol*. 2016;9(1):46–56.
9. Steinberg M, McBride WH, Vlashi E, et al. National Institutes of Health funding in radiation oncology: A snapshot. *Int J Radiat Oncol Biol Phys*. 2013;86(2):234–240.
10. Zeman EM, Dynlacht JR, Rosenstein BS, et al. Toward a national consensus: Teaching radiobiology to radiation oncology residents. *Int J Radiat Oncol Biol Phys*. 2002;54(3):861–872.
11. Rosenstein BS, Held KD, Rockwell S, et al. American Society for Radiation Oncology (ASTRO) survey of radiation biology educators in U.S. and Canadian radiation oncology residency programs. *Int J Radiat Oncol Biol Phys*. 2009;75(3):896–905.
12. Franken NA, Rodermond HM, Stap J, et al. Clonogenic assay of cells *in vitro*. *Nat Protoc*. 2006;1(5):2315–2319.
13. Tiwana GS, Prevo R, Buffa FM, et al. Identification of vitamin B1 metabolism as a tumor-specific radiosensitizing pathway using a high-throughput colony formation screen. *Oncotarget*. 2015;6(8):5978–5989.
14. Higgins GS, Prevo R, Lee YF, et al. A small interfering RNA screen of genes involved in DNA repair identifies tumor-specific radiosensitization by POLQ knockdown. *Cancer Res*. 2010;70(7):2984–2993.
15. Labay E, Mauceri HJ, Efimova EV, et al. Repurposing cephalosporin antibiotics as pro-senescent radiosensitizers. *Oncotarget*. In press.
16. Wei D, Parsels LA, Karnak D, et al. Inhibition of protein phosphatase 2A radiosensitizes pancreatic cancers by modulating CDC25C/CDK1 and homologous recombination repair. *Clin Cancer Res*. 2013;19(16):4422–4432.
17. Hatano K, Kumar B, Zhang Y, et al. A functional screen identifies miRNAs that inhibit DNA repair and sensitize prostate cancer cells to ionizing radiation. *Nucleic Acids Res*. 2015;43(8):4075–4086.
18. Hidalgo M, Amant F, Biankin AV, et al. Patient-derived xenograft models: An emerging platform for translational cancer research. *Cancer Discov*. 2014;4(9):998–1013.
19. Stone HB, Peters LJ, Milas L. Effect of host immune capability on radiocurability and subsequent transplantability of a murine fibrosarcoma. *J Natl Cancer Inst*. 1979;63(5):1229–1235.
20. Perez BA, Ghafoori AP, Lee CL, et al. Assessing the radiation response of lung cancer with different gene mutations using genetically engineered mice. *Front Oncol*. 2013;3:72.

21. Moding EJ, Lee CL, Castle KD, et al. Atm deletion with dual recombinase technology preferentially radiosensitizes tumor endothelium. *J Clin Invest*. 2014;124(8):3325–3338.
22. Moding EJ, Castle KD, Perez BA, et al. Tumor cells, but not endothelial cells, mediate eradication of primary sarcomas by stereotactic body radiation therapy. *Sci Transl Med*. 2015;7(278):278ra34.
23. Zhang M, Qiu Q, Li Z, et al. HIF-1 alpha regulates the response of primary sarcomas to radiation therapy through a cell autonomous mechanism. *Radiat Res*. 2015;183(6):594–609.
24. Herter-Sprie GS, Koyama S, Korideck H, et al. Synergy of radiotherapy and PD-1 blockade in Kras-mutant lung cancer. *JCI Insight*. 2016;1(9):e87415.
25. Zhao J, Kim KA, De Vera J, et al. R-Spondin1 protects mice from chemotherapy or radiation-induced oral mucositis through the canonical Wnt/beta-catenin pathway. *Proc Natl Acad Sci U S A*. 2009;106(7):2331–2336.
26. Engelke CG, Parsels LA, Qian Y, et al. Sensitization of pancreatic cancer to chemoradiation by the Chk1 inhibitor MK8776. *Clin Cancer Res*. 2013;19(16):4412–4421.
27. Medin PM, Foster RD, van der Kogel AJ, et al. Spinal cord tolerance to reirradiation with single-fraction radiosurgery: A swine model. *Int J Radiat Oncol Biol Phys*. 2012;83(3):1031–1037.
28. Clermont T, Leblanc AK, Adams WH, et al. Radiotherapy-induced myelosuppression in dogs: 103 cases (2002-2006). *Vet Comp Oncol*. 2012;10(1):24–32.
29. LeBlanc AK, Breen M, Choyke P, et al. Perspectives from man's best friend: National Academy of Medicine's Workshop on Comparative Oncology. *Sci Transl Med*. 2016;8(324):324ps5.
30. Eke I, Cordes N. Radiobiology goes 3D: How ECM and cell morphology impact on cell survival after irradiation. *Radiother Oncol*. 2011;99(3):271–278.
31. Zhou BB, Bartek J. Targeting the checkpoint kinases: Chemosensitization versus chemoprotection. *Nat Rev Cancer*. 2004;4(3):216–225.
32. Matsuoka S, Ballif BA, Smogorzewska A, et al. ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science*. 2007;316(5828):1160–1166.
33. Golding SE, Rosenberg E, Valerie N, et al. Improved ATM kinase inhibitor KU-60019 radiosensitizes glioma cells, compromises insulin, AKT and ERK prosurvival signaling, and inhibits migration and invasion. *Mol Cancer Ther*. 2009;8(10):2894–2902.
34. Biddlestone-Thorpe L, Sajjad M, Rosenberg E, et al. ATM kinase inhibition preferentially sensitizes p53-mutant glioma to ionizing radiation. *Clin Cancer Res*. 2013;19(12):3189–3200.
35. Batey MA, Zhao Y, Kyle S, et al. Preclinical evaluation of a novel ATM inhibitor, KU59403, in vitro and in vivo in p53 functional and dysfunctional models of human cancer. *Mol Cancer Ther*. 2013;12(6):959–967.
36. Pike KG. Abstract 4859: Identifying high quality, potent and selective inhibitors of ATM kinase: Discovery of AZD0156. *Cancer Res*. 2016;76(14 supplement):4859–4859.
37. Fokas E, Prevo R, Pollard JR, et al. Targeting ATR in vivo using the novel inhibitor VE-822 results in selective sensitization of pancreatic tumors to radiation. *Cell Death Dis*. 2012;3:e441.
38. Hall AB, Newsome D, Wang Y, et al. Potentiation of tumor responses to DNA damaging therapy by the selective ATR inhibitor VX-970. *Oncotarget*. 2014;5(14):5674–5685.
39. Jones CD, Blades K, Foote KM, et al. Abstract 2348: Discovery of AZD6738, a potent and selective inhibitor with the potential to test the clinical efficacy of ATR kinase inhibition in cancer patients. *Cancer Res*. 2013;73(8 supplement):2348–2348.
40. Guichard SM, Brown E, Odedra R, et al. Abstract 3343: The pre-clinical in vitro and in vivo activity of AZD6738: A potent and selective inhibitor of ATR kinase. *Cancer Res*. 2013;73(8 supplement):3343–3343.
41. De Craene B, Berx G. Regulatory networks defining EMT during cancer initiation and progression. *Nat Rev Cancer*. 2013;13(2):97–110.
42. Bao S, Wu Q, McLendon RE, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature*. 2006;444(7120):756–760.
43. Baumann M, Krause M, Hill R. Exploring the role of cancer stem cells in radioresistance. *Nat Rev Cancer*. 2008;8(7):545–554.
44. Zhang P, Wei Y, Wang L, et al. ATM-mediated stabilization of ZEB1 promotes DNA damage response and radioresistance through CHK1. *Nat Cell Biol*. 2014;16(9):864–875.
45. Zhang P, Wang L, Rodriguez-Aguayo C, et al. miR-205 acts as a tumour radiosensitizer by targeting ZEB1 and Ubc13. *Nat Commun*. 2014;5:5671.
46. Fokas E, Im JH, Hill S, et al. Dual inhibition of the PI3K/mTOR pathway increases tumor radiosensitivity by normalizing tumor vasculature. *Cancer Res*. 2012;72(1):239–248.
47. Toulany M, Rodemann HP. Phosphatidylinositol 3-kinase/Akt signaling as a key mediator of tumor cell responsiveness to radiation. *Semin Cancer Biol*. 2015;35:180–190.
48. Feron O. Pyruvate into lactate and back: From the Warburg effect to symbiotic energy fuel exchange in cancer cells. *Radiother Oncol*. 2009;92(3):329–333.
49. Kimmelman AC. Metabolic dependencies in RAS-driven cancers. *Clin Cancer Res*. 2015;21(8):1828–1834.
50. Hsieh AL, Walton ZE, Altman BJ, et al. MYC and metabolism on the path to cancer. *Semin Cell Dev Biol*. 2015;43:11–21.
51. Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. *Nat Rev Cancer*. 2011;11(2):85–95.
52. Menon SS, Uppal M, Randhawa S, et al. Radiation metabolomics: Current status and future directions. *Front Oncol*. 2016;6:20.
53. Singh SK, Hawkins C, Clarke ID, et al. Identification of human brain tumour initiating cells. *Nature*. 2004;432(7015):396–401.
54. Al-Hajj M, Wicha MS, Benito-Hernandez A, et al. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci U S A*. 2003;100(7):3983–3988.
55. Vlashi E, Lagadec C, Vergnes L, et al. Metabolic differences in breast cancer stem cells and differentiated progeny. *Breast Cancer Res Treat*. 2014;146(3):525–534.
56. Vlashi E, Lagadec C, Vergnes L, et al. Metabolic state of glioma stem cells and nontumorigenic cells. *Proc Natl Acad Sci U S A*. 2011;108(38):16062–16067.
57. Phillips TM, McBride WH, Pajonk F. The response of CD24(-/low)/CD44+ breast cancer-initiating cells to radiation. *J Natl Cancer Inst*. 2006;98(24):1777–1785.
58. Lagadec C, Dekmezian C, Bauche L, et al. Oxygen levels do not determine radiation survival of breast cancer stem cells. *PLoS One*. 2012;7(3):e34545.
59. Diehn M, Cho RW, Lobo NA, et al. Association of reactive oxygen species levels and radioresistance in cancer stem cells. *Nature*. 2009;458(7239):780–783.
60. Kroonen J, Nassen J, Boulanger YG, et al. Human glioblastoma-initiating cells invade specifically the subventricular zones and olfactory bulbs of mice after striatal injection. *Int J Cancer*. 2011;129(3):574–585.
61. Evers P, Lee PP, DeMarco J, et al. Irradiation of the potential cancer stem cell niches in the adult brain improves progression-free survival of patients with malignant glioma. *BMC Cancer*. 2010;10:384.
62. Chaffer CL, Brueckmann I, Scheel C, et al. Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state. *Proc Natl Acad Sci U S A*. 2011;108(19):7950–7955.
63. Vissink A, Mitchell JB, Baum BJ, et al. Clinical management of salivary gland hypofunction and xerostomia in head-and-neck cancer patients: Successes and barriers. *Int J Radiat Oncol Biol Phys*. 2010;78(4):983–991.
64. Lombaert IM, Brunsting JF, Wierenga PK, et al. Cytokine treatment improves parenchymal and vascular damage of salivary glands after irradiation. *Clin Cancer Res*. 2008;14(23):7741–7750.
65. Knox SM, Lombaert IM, Haddox CL, et al. Parasympathetic stimulation improves epithelial organ regeneration. *Nat Commun*. 2013;4:1494.
66. Lombaert IM, Brunsting JF, Wierenga PK, et al. Keratinocyte growth factor prevents radiation damage to salivary glands by expansion of the stem/progenitor pool. *Stem Cells*. 2008;26(10):2595–2601.
67. Lombaert IM, Abrams SR, Li L, et al. Combined KIT and FGFR2b signaling regulates epithelial progenitor expansion during organogenesis. *Stem Cell Reports*. 2013;1(6):604–619.
68. Xiao N, Lin Y, Cao H, et al. Neurotrophic factor GDNF promotes survival of salivary stem cells. *J Clin Invest*. 2014;124(8):3364–3377.
69. Zeidan YH, Xiao N, Cao H, et al. Botulinum toxin confers radioprotection in murine salivary glands. *Int J Radiat Oncol Biol Phys*. 2016;94(5):1190–1197.
70. van Luijk P, Pringle S, Deasy JO, et al. Sparing the region of the salivary gland containing stem cells preserves saliva production after radiotherapy for head and neck cancer. *Sci Transl Med*. 2015;7(305):305ra147.
71. Formenti SC, Demaria S. Combining radiotherapy and cancer immunotherapy: A paradigm shift. *J Natl Cancer Inst*. 2013;105(4):256–265.
72. Demaria S, Ng B, Devitt ML, et al. Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. *Int J Radiat Oncol Biol Phys*. 2004;58(3):862–870.
73. Dewan MZ, Galloway AE, Kawashima N, et al. Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. *Clin Cancer Res*. 2009;15(17):5379–5388.
74. Twyman-Saint Victor C, Rech AJ, Maity A, et al. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. *Nature*. 2015;520(7547):373–377.
75. Postow MA, Callahan MK, Barker CA, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. *N Engl J Med*. 2012;366(10):925–931.
76. Grimaldi AM, Simeone E, Giannarelli D, et al. Abscopal effects of radiotherapy on advanced melanoma patients who progressed after ipilimumab immunotherapy. *Oncoimmunology*. 2014;3:e28780.
77. Chandra RA, Wilhite TJ, Balboni TA, et al. A systematic evaluation of abscopal responses following radiotherapy in patients with metastatic melanoma treated with ipilimumab. *Oncoimmunology*. 2015;4(11):e1046028.
78. Golden EB, Demaria S, Schiff PB, et al. An abscopal response to radiation and ipilimumab in a patient with metastatic non-small cell lung cancer. *Cancer Immunol Res*. 2013;1(6):365–372.
79. Golden EB, Chhabra A, Chachoua A, et al. Local radiotherapy and granulocyte-macrophage colony-stimulating factor to generate abscopal responses in patients with metastatic solid tumours: A proof-of-principle trial. *Lancet Oncol*. 2015;16(7):795–803.
80. Kwilas AR, Donahue RN, Bernstein MB, et al. In the field: Exploiting the untapped potential of immunogenic modulation by radiation in combination with immunotherapy for the treatment of cancer. *Front Oncol*. 2012;2:104.
81. Burnette B, Fu YX, Weichselbaum RR. The confluence of radiotherapy and immunotherapy. *Front Oncol*. 2012;2:143.

82. Schaue D, Micewicz ED, Ratikan JA, et al. Radiation and inflammation. *Semin Radiat Oncol*. 2015;25(1):4–10.
83. McBride WH, Chiang CS, Olson JL, et al. A sense of danger from radiation. *Radiat Res*. 2004;162(1):1–19.
84. Reits EA, Hodge JW, Herberts CA, et al. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. *J Exp Med*. 2006;203(5):1259–1271.
85. Ratikan JA, Micewicz ED, Xie MW, et al. Radiation takes its toll. *Cancer Lett*. 2015;368(2):238–245.
86. Schaue D, Comin-Anduix B, Ribas A, et al. T-cell responses to survivin in cancer patients undergoing radiation therapy. *Clin Cancer Res*. 2008;14(15):4883–4890.
87. Vanpouille-Box C, Diamond JM, Pilones KA, et al. TGFbeta is a master regulator of radiation therapy-induced antitumor immunity. *Cancer Res*. 2015;75(11):2232–2242.
88. Haddow A, Alexander P. An immunological method of increasing the sensitivity of primary sarcomas to local irradiation with x rays. *Lancet*. 1964;1(7331):452–457.
89. Demaria S, Coleman CN, Formenti SC. Radiotherapy: Changing the game in immunotherapy. *Trends Cancer*. 2016;2(6):286–294.
90. Gandhi SJ, Minn AJ, Vonderheide RH, et al. Awakening the immune system with radiation: Optimal dose and fractionation. *Cancer Lett*. 2015;368(2):185–190.
91. Vanpouille-Box C, Alard A, Aryankalayil MJ, et al. DNA exonuclease Trex1 regulates radiotherapy-induced tumour immunogenicity. *Nat Commun*. 2017;8:15618.
92. Jaffray DA. Image-guided radiotherapy: From current concept to future perspectives. *Nat Rev Clin Oncol*. 2012;9(12):688–699.
93. Blank CU, Haanen JB, Ribas A, et al. Cancer immunology. The "cancer immunogram." *Science*. 2016;352(6286):658–660.
94. Horsman MR, Vaupel P. Pathophysiological basis for the formation of the tumor microenvironment. *Front Oncol*. 2016;6:66.
95. Vaupel P, Hoeckel M. Predictive power of the tumor oxygenation status. *Adv Exp Med Biol*. 1999;471:533–539.
96. Hall EJ, Giaccia AJ. *Chapter 6: Oxygen Effect and Reoxygenation*. Philadelphia: Lippincott Williams & Wolters Kluwer; 2012.
97. Vaupel P. Metabolic microenvironment of tumor cells: A key factor in malignant progression. *Exp Oncol*. 2010;32(3):125–127.
98. Lin A, Maity A. Molecular pathways: A novel approach to targeting hypoxia and improving radiotherapy efficacy via reduction in oxygen demand. *Clin Cancer Res*. 2015;21(9):1995–2000.
99. Wilson WR, Hay MP. Targeting hypoxia in cancer therapy. *Nat Rev Cancer*. 2011;11(6):393–410.
100. Janssens GO, Rademakers SE, Terhaard CH, et al. Improved recurrence-free survival with ARCON for anemic patients with laryngeal cancer. *Clin Cancer Res*. 2014;20(5):1345–1354.
101. Secomb TW, Hsu R, Dewhirst MW. Synergistic effects of hyperoxic gas breathing and reduced oxygen consumption on tumor oxygenation: A theoretical model. *Int J Radiat Oncol Biol Phys*. 2004;59(2):572–578.
102. Koritzinsky M. Metformin: A novel biological modifier of tumor response to radiation therapy. *Int J Radiat Oncol Biol Phys*. 2015;93(2):454–464.
103. Jain RK. Antiangiogenesis strategies revisited: From starving tumors to alleviating hypoxia. *Cancer Cell*. 2014;26(5):605–622.
104. Kioi M, Vogel H, Schultz G, et al. Inhibition of vasculogenesis, but not angiogenesis, prevents the recurrence of glioblastoma after irradiation in mice. *J Clin Invest*. 2010;120(3):694–705.
105. Chaudary N, Pintilie M, Jelveh S, et al. Plerixafor improves primary tumor response and reduces metastases in cervical cancer treated with radio-chemotherapy. *Clin Cancer Res*. 2017;23(5):1242–1249.
106. Koonce NA, Levy J, Hardee ME, et al. Targeting artificial tumor stromal targets for molecular imaging of tumor vascular hypoxia. *PLoS One*. 2015;10(8):e0135607.
107. Terman DS, Viglianti BL, Zennadi R, et al. Sickle erythrocytes target cytotoxics to hypoxic tumor microvessels and potentiate a tumoricidal response. *PLoS One*. 2013;8(1):e52543.
108. Kotb S, Detappe A, Lux F, et al. Gadolinium-based nanoparticles and radiation therapy for multiple brain melanoma metastases: Proof of concept before phase I trial. *Theranostics*. 2016;6(3):418–427.
109. Dorsey JF, Sun L, Joh DY, et al. Gold nanoparticles in radiation research: Potential applications for imaging and radiosensitization. *Transl Cancer Res*. 2013;2(4):280–291.
110. Koonce NA, Quick CM, Hardee ME, et al. Combination of gold nanoparticle-conjugated tumor necrosis factor-alpha and radiation therapy results in a synergistic antitumor response in murine carcinoma models. *Int J Radiat Oncol Biol Phys*. 2015;93(3):588–596.
111. Song CW, Lee YJ, Griffin RJ, et al. Indirect tumor cell death after high-dose hypofractionated irradiation: Implications for stereotactic body radiation therapy and stereotactic radiation surgery. *Int J Radiat Oncol Biol Phys*. 2015;93(1):166–172.
112. Maeda A, Leung MK, Conroy L, et al. In vivo optical imaging of tumor and microvascular response to ionizing radiation. *PLoS One*. 2012;7(8):e42133.
113. Jani A, Shaikh F, Barton S, et al. High-dose, single-fraction irradiation rapidly reduces tumor vasculature and perfusion in a xenograft model of neuroblastoma. *Int J Radiat Oncol Biol Phys*. 2016;94(5):1173–1180.
114. Malik R, Lelkes PI, Cukierman E. Biomechanical and biochemical remodeling of stromal extracellular matrix in cancer. *Trends Biotechnol*. 2015;33(4):230–236.
115. Park CC, Zhang HJ, Yao ES, et al. Beta1 integrin inhibition dramatically enhances radiotherapy efficacy in human breast cancer xenografts. *Cancer Res*. 2008;68(11):4398–4405.
116. Nam JM, Onodera Y, Bissell MJ, et al. Breast cancer cells in three-dimensional culture display an enhanced radioresponse after coordinate targeting of integrin alpha5beta1 and fibronectin. *Cancer Res*. 2010;70(13):5238–5248.
117. Eke I, Zscheppang K, Dickreuter E, et al. Simultaneous beta1 integrin-EGFR targeting and radiosensitization of human head and neck cancer. *J Natl Cancer Inst*. 2015;107(2):dju419.
118. Sethi T, Rintoul RC, Moore SM, et al. Extracellular matrix proteins protect small cell lung cancer cells against apoptosis: A mechanism for small cell lung cancer growth and drug resistance in vivo. *Nat Med*. 1999;5(6):662–668.
119. Lin CH, Pelissier FA, Zhang H, et al. Microenvironment rigidity modulates responses to the HER2 receptor tyrosine kinase inhibitor lapatinib via YAP and TAZ transcription factors. *Mol Biol Cell*. 2015;26(22):3946–3953.
120. Eke I, Schneider L, Forster C, et al. EGFR/JIP-4/JNK2 signaling attenuates cetuximab-mediated radiosensitization of squamous cell carcinoma cells. *Cancer Res*. 2013;73(1):297–306.
121. Halder G, Dupont S, Piccolo S. Transduction of mechanical and cytoskeletal cues by YAP and TAZ. *Nat Rev Mol Cell Biol*. 2012;13(9):591–600.
122. Friedland JC, Lee MH, Boettiger D. Mechanically activated integrin switch controls alpha5beta1 function. *Science*. 2009;323(5914):642–644.
123. Kai F, Laklai H, Weaver VM. Force matters: Biomechanical regulation of cell invasion and migration in disease. *Trends Cell Biol*. 2016;26(7):486–497.
124. Levental KR, Yu H, Kass L, et al. Matrix crosslinking forces tumor progression by enhancing integrin signaling. *Cell*. 2009;139(5):891–906.
125. Dupont S, Morsut L, Aragona M, et al. Role of YAP/TAZ in mechanotransduction. *Nature*. 2011;474(7350):179–183.
126. La Thangue NB, Kerr DJ. Predictive biomarkers: A paradigm shift towards personalized cancer medicine. *Nat Rev Clin Oncol*. 2011;8(10):587–596.
127. Baumann M, Krause M, Overgaard J, et al. Radiation oncology in the era of precision medicine. *Nat Rev Cancer*. 2016;16(4):234–249.
128. Yard B, Chie EK, Adams DJ, et al. Radiotherapy in the era of precision medicine. *Semin Radiat Oncol*. 2015;25(4):227–236.
129. Willers H, Held KD. Introduction to clinical radiation biology. *Hematol Oncol Clin North Am*. 2006;20(1):1–24.
130. Willers H, Dahm-Daphi J, Powell SN. Repair of radiation damage to DNA. *Br J Cancer*. 2004;90(7):1297–1301.
131. Evans JR, Zhao SG, Chang SL, et al. Patient-level DNA damage and repair pathway profiles and prognosis after prostatectomy for high-risk prostate cancer. *JAMA Oncol*. 2016;2(4):471–480.
132. Al-Ahmadie H, Iyer G, Hohl M, et al. Synthetic lethality in ATM-deficient RAD50-mutant tumors underlies outlier response to cancer therapy. *Cancer Discov*. 2014;4(9):1014–1021.
133. Pitroda SP, Pashtan IM, Logan HL, et al. DNA repair pathway gene expression score correlates with repair proficiency and tumor sensitivity to chemotherapy. *Sci Transl Med*. 2014;6(229):229ra42.
134. Fraser M, Harding SM, Zhao H, et al. MRE11 promotes AKT phosphorylation in direct response to DNA double-strand breaks. *Cell Cycle*. 2011;10(13):2218–2232.
135. Kan C, Zhang J. BRCA1 mutation: A predictive marker for radiation therapy? *Int J Radiat Oncol Biol Phys*. 2015;93(2):281–293.
136. Pearl LH, Schierz AC, Ward SE, et al. Therapeutic opportunities within the DNA damage response. *Nat Rev Cancer*. 2015;15(3):166–180.
137. Chae YK, Anker JF, Carneiro BA, et al. Genomic landscape of DNA repair genes in cancer. *Oncotarget*. 2016;7(17):23312–23321.
138. Jackson SP, Helleday T. DNA REPAIR. Drugging DNA repair. *Science*. 2016;352(6290):1178–1179.
139. Rieckmann T, Tribius S, Grob TJ, et al. HNSCC cell lines positive for HPV and p16 possess higher cellular radiosensitivity due to an impaired DSB repair capacity. *Radiother Oncol*. 2013;107(2):242–246.
140. Kimple RJ, Smith MA, Blitzer GC, et al. Enhanced radiation sensitivity in HPV-positive head and neck cancer. *Cancer Res*. 2013;73(15):4791–4800.
141. Chera BS, Amdur RJ, Tepper J, et al. Phase 2 trial of de-intensified chemoradiation therapy for favorable-risk human papillomavirus-associated oropharyngeal squamous cell carcinoma. *Int J Radiat Oncol Biol Phys*. 2015;93(5):976–985.
142. Eschrich SA, Pramana J, Zhang H, et al. A gene expression model of intrinsic tumor radiosensitivity: Prediction of response and prognosis after chemoradiation. *Int J Radiat Oncol Biol Phys*. 2009;75(2):489–496.
143. Willers H, Gheorghiu L, Liu Q, et al. DNA damage response assessments in human tumor samples provide functional biomarkers of radiosensitivity. *Semin Radiat Oncol*. 2015;25(4):237–250.
144. Menegakis A, von Neubeck C, Yaromina A, et al. gammaH2AX assay in ex vivo irradiated tumour specimens: A novel method to determine tumour radiation sensitivity in patient-derived material. *Radiother Oncol*. 2015;116(3):473–479.

145. Cornelissen B, Kersemans V, Darbar S, et al. Imaging DNA damage in vivo using gammaH2AX-targeted immunoconjugates. *Cancer Res*. 2011;71(13):4539–4549.
146. Lalonde E, Ishkanian AS, Sykes J, et al. Tumour genomic and microenvironmental heterogeneity for integrated prediction of 5-year biochemical recurrence of prostate cancer: A retrospective cohort study. *Lancet Oncol*. 2014;15(13):1521–1532.
147. Haber DA, Velculescu VE. Blood-based analyses of cancer: Circulating tumor cells and circulating tumor DNA. *Cancer Discov*. 2014;4(6):650–661.
148. Chaudhuri AA, Binkley MS, Osmundson EC, et al. Predicting radiotherapy responses and treatment outcomes through analysis of circulating tumor DNA. *Semin Radiat Oncol*. 2015;25(4):305–312.
149. Lin JC, Wang WY, Chen KY, et al. Quantification of plasma Epstein-Barr virus DNA in patients with advanced nasopharyngeal carcinoma. *N Engl J Med*. 2004;350(24):2461–2470.
150. Newman AM, Bratman SV, To J, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. *Nat Med*. 2014;20(5):548–554.
151. Scott JG, Berglund A, Schell MJ, et al. A genome-based model for adjusting radiotherapy dose (GARD): A retrospective, cohort-based study. *Lancet Oncol*. 2017;18(2):202–211.
152. Zhao SG, Chang SL, Spratt DE, et al. Development and validation of a 24-gene predictor of response to postoperative radiotherapy in prostate cancer: A matched, retrospective analysis. *Lancet Oncol*. 2016;17(11):1612–1620.
153. Bossuyt PM, Reitsma JB, Bruns DE, et al. Towards complete and accurate reporting of studies of diagnostic accuracy: The STARD initiative. Standards for Reporting of Diagnostic Accuracy. *Clin Chem*. 2003;49(1):1–6.
154. McShane LM, Altman DG, Sauerbrei W, et al. REporting recommendations for tumour MARKer prognostic studies (REMARK). *Br J Cancer*. 2005;93(4):387–391.