CURRENT STATUS and RECOMMENDATIONS for the FUTURE of RESEARCH, TEACHING and TESTING in the BIOLOGICAL SCIENCES of RADIATION ONCOLOGY

REPORT OF THE AMERICAN SOCIETY FOR RADIATION ONCOLOGY CANCER BIOLOGY/RADIATION BIOLOGY TASK FORCE
Acknowledgements

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Background

In early 2011, a dialogue was initiated within the Board of Directors (BOD) of the American Society for Radiation Oncology (ASTRO) regarding the future of the basic sciences of the specialty. The decision was primarily focused on the current state and potential future direction of basic research within radiation oncology. After consideration of the complexity of the issues involved and the precise nature of the undertaking, in August 2011, the BOD empanelled a Cancer Biology/Radiation Biology Task Force (TF). The TF was charged with developing an accurate snapshot of the current state of basic (pre-clinical) research in radiation oncology from the perspective of relevance to the modern clinical practice of radiation oncology, as well as the education of our trainees and attending physicians in the biological sciences. The TF was further charged with developing suggestions in critical areas of biological basic research that would maintain and further construct the scientific foundation and vitality of radiation oncology as an independent and vibrant medical specialty. It was not within the scope of service of the TF to consider the quality of ongoing research efforts within the broader radiation oncology space, to presume to consider their future potential, or to discourage in any way the investigators committed to areas of interest other than those targeted. The TF charge specifically precluded consideration of research issues related to technology, physics, or clinical investigations.
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The essence of progress in any primary medical specialty involves the constant incorporation of relevant basic science observations, drug and technological discoveries, and clinical research findings, into the clinical armamentarium. From the outset of radiation oncology as a clinical discipline in the early 1900’s, this scientific rigor has moved the specialty inexorably forward. During much of the 20th century, biological findings and knowledge derived from irradiation of animals, pathological findings in human normal and tumor-involved tissues and organs, and empirical observations of superficial tumors, were predominant in establishing the biological principles of the specialty. These were implemented into the lexicon of the specialty relating to the volume of tissue irradiated and associated morbidity in addition to altered dose fractionation and protraction. In the late 1960’s and 1970’s, there was an increased availability of medical linear accelerators, radiation treatment planning computers, CT scanners and the use of multi-agent systemic chemotherapy. This led to the primary focus of radiation research evolving towards strategies to increase radiation dose deposition to tumor tissue and tumor sensitization, limitation of doses to normal tissues and normal tissue protection, and optimizing interactions between a variety of chemotherapy combinations and schedules with external beam radiation. Following the turn of the century, scientific investigations focused increasingly on molecular processes inherent in normal and cancer cells; however radiation-related research has been lagging in the adoption of these new findings and paradigms, specifically as to how they impact the effect of radiation on normal and pathologic tissues. Within the “radiation research” community there has also been a debate regarding the precise nature of the field, with a narrow definition citing that research is carried out within and under the auspices of departments of radiation oncology. Additionally, a broader definition recognizes radiation as being one of the most potent modulators of cellular activity, and that any research with radiation, or using radiation as a biological modifier, is “radiation research.”

Following the turn of the century, scientific investigations focused increasingly on molecular processes inherent in normal and cancer cells; however radiation-related research has been lagging in the adoption of these new findings and paradigms, specifically as to how they impact the effect of radiation on normal and pathologic tissues.
In the ensuing decades, various authors have attempted to develop a sense of the state of investigation and funding in radiation research. These reports typically focused on the number of NIH-funded projects, the classification (e.g. investigator-initiated RO-1, etc.), size, and duration of funding of those projects. These efforts rarely included reviews of the nature of the actual science involved, cohesiveness to other projects in the field, or their potential impact on the specialty. Presumably, the manuscripts were developed to energize scientists within the profession, and direct specialty-based policy-makers in outreach to Federal funding agencies and advocacy groups. A recurring theme in these publications was the “undercounting” of research to radiation oncology departments due to the lack of specialty-specific reporting of research grants and contracts.12

The purpose defined by the ASTRO BOD for the TF activities was to consider all of these issues in context with changes in the funding and delivery of healthcare in general, and radiation oncology in particular. The TF was to consider the current state of the radiation research enterprise, and to suggest a coherent strategy for future research directions and strategies that might be anticipated to be most beneficial to the specialty.

**Material and Methods**

The TF members were appointed by the ASTRO BOD, and consisted of senior clinicians, clinical investigators and basic research scientists, as well as a cadre of early and mid-career basic and translational science investigators (see Appendix I). An organizational meeting of the TF was convened at the 2011 Annual Meeting of the Society, following activities which were carried out by individual TF members, conference calls, and electronic communication, all with the continuous support of ASTRO staff.

TF members and staff developed an extensive survey instrument, which was launched on February 15, 2012 and closed on March 30, 2012. The survey was sent electronically to over 400 Radiation Research Society (RRS) members and approximately 1,200 ASTRO members who had identified themselves as having an interest in biology with MD, MD/PhD, or PhD credentials. The survey was designed to develop a snapshot of current research, research funding, perceptions of the radiation research enterprise, and “trend spotting” for the future. The survey instrument was not designed to seek precision in quantification or statistical significance therefore; responses were broadly aggregated to develop a sense of the responses. Of the 465 responses, 395 (25%) were viewed as valid responses to the survey. Responses were collated and reviewed by ASTRO staff and TF members. Following data cleaning (e.g., recoded open text responses), an initial survey report that included frequencies and descriptive statistics for selected survey questions was developed (see Appendix II).

In addition to the survey instrument used for individuals directly involved in the radiation oncology research and clinical enterprise, telephone surveys were conducted with basic research, clinical, and oncology organizational thought leaders who had been identified by TF members and survey respondents. These individuals were felt to be knowledgeable regarding trends within basic oncology research and funding, and were not personally involved with radiation research efforts. The interviews were prescheduled; interviewees were briefed on the reason for an interview, as well as the issues to be discussed. Interviews were carried out by single ASTRO staff members using a script developed by the TF. Trial runs of the script and interviews were carried out with cooperating National Institute of Health (NIH) staff investigators. The intent of these focused interviews was to gain insight into the perceptions of the current state of basic biological radiation research as held by those not identified with the enterprise (see Appendix III).

TF members and ASTRO staff carried out an extensive literature review, a review of previous Association of Residents in Radiation Oncology (ARRO) member surveys, previous research reports, a review of publicly available data from the NIH and National Cancer Institute (NCI) grant awards databases, Accreditation Council on Graduate Medical Education Essentials for training in radiation oncology, as well as the American Board of Radiology (ABR) study guide for initial certification (see Appendix IV and V). Following completion of the review and data collation process, TF members employed an extensive, multi-layered consensus development process to determine major scientific topics for
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subsequent consideration, and expanded text development. To maintain editorial consistency, several topics were consolidated and several suggested topics that were outside of the TF charge, e.g. physics or technology-based, were eliminated from consideration. Section writers were either volunteers from the TF membership, or non-TF members invited to contribute due to their individual interest or expertise. To ensure that critical areas of research were not overlooked, TF members were encouraged to add material related to areas of interest not identified by the consensus-development process. Individual scientific sections were subjected to a second level of TF editorial and content review, and a third level of TF editorial review. Upon completion of a draft of the collated document, the entire TF carried out an editorial review and subsequently, independent non-TF review was carried out by several nationally recognized senior scientists to ensure accuracy, completeness and fairness of the TF recommendations. Relevant comments and suggestions made by external reviewers were incorporated into the text. The TF finalized and submitted its report to the ASTRO BOD for review, comment, and approval; this was completed on August 13th, 2013.

REFERENCES

To determine the current state of Radiation Oncology Biology funding, two methods were employed to gather data. The first was a query from the ASTRO Government relations staff to congress about actual Radiation Oncology funding levels, and the second was a review of the publicly available grant system database. Although Congress has been a long-standing supporter of biomedical research, funding levels have been on the decline in recent years. Despite many successes, progress in radiation oncology research efforts have likely been hampered due to Federal funding not keeping pace. At ASTRO’s request, Rep. Denny Rehberg (R-Mont.), chairman of the House Appropriations Health Subcommittee, submitted a written request in 2012 for the NIH and NCI to provide a report of the federal funding directed to radiation therapy specific projects for FY2010, FY2011 and FY2012.1

In response to this Congressional request, NIH acknowledged that less than one percent of the total NIH budget in Fiscal Years 2010 and 2011 was spent on radiation oncology research, and just over four percent of NCI’s total budget was spent on radiation oncology-specific projects in Fiscal Years 2010 and 2011. However, this report was not able to differentiate spending on clinical trials, physics research and biological research. A more recent review by Steinberg et al. corroborated these findings.2

To differentiate biological research from clinical trials and physics research, all Radiation Oncology grants listed on the NIH Report website3 were hand-curated, separating the biology grants from the clinical and physics grants. Furthermore, the biology grants were then subdivided by research topic. As shown in Figure 1 below, the three most funded subgroups were: Radiosensitizers, Normal Tissue, and Tumor Microenvironment.4

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### FIGURE 1: Areas of Research Specialization

<table>
<thead>
<tr>
<th>Area</th>
<th>Funding Percentage</th>
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<tbody>
<tr>
<td>Tumor Microenvironment</td>
<td>18%</td>
</tr>
<tr>
<td>Normal Tissue</td>
<td>15%</td>
</tr>
<tr>
<td>Radiosensitizer</td>
<td>15%</td>
</tr>
<tr>
<td>Cell Cycle/Signaling</td>
<td>12%</td>
</tr>
<tr>
<td>RadiolImmuno Therapy</td>
<td>10%</td>
</tr>
<tr>
<td>Systemic Therapy-Targeted</td>
<td>5%</td>
</tr>
<tr>
<td>DNA Damage</td>
<td>5%</td>
</tr>
<tr>
<td>Carcinogenesis</td>
<td>3%</td>
</tr>
<tr>
<td>Cancer Stem Cells</td>
<td>3%</td>
</tr>
<tr>
<td>Biomarkers/Radiogenomics</td>
<td>3%</td>
</tr>
<tr>
<td>Immunology</td>
<td>3%</td>
</tr>
<tr>
<td>Radioprotectors</td>
<td>2%</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>2%</td>
</tr>
<tr>
<td>Hyperthermia RT</td>
<td>1%</td>
</tr>
<tr>
<td>Protons</td>
<td>1%</td>
</tr>
<tr>
<td>Nanotherapeutics</td>
<td>1%</td>
</tr>
<tr>
<td>Radiochemistry</td>
<td>1%</td>
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</table>
Proposed areas of scientific concentration

Selection of the areas of scientific investigation (discussed in detail below) represented an iterative process that included TF members and non-TF basic scientists, clinician-scientists, and clinicians. Suggested topics that were determined to be more appropriately related to pure clinical, clinical/translational, physics, or technology, were eliminated from consideration due to being beyond the scope of the TF mission. No attempt was made to develop a catalogue of current areas of investigation in the aforementioned areas within the active radiation research enterprise. Additionally, no attempts were made to carry out an extensive evaluation of any ongoing projects or laboratory resources. Topics are not listed in any order of priority, nor was that issue considered by the TF. TF scientific recommendations were not based on research endeavors that held the potential for greater prospects of successful funding from the NCI, as might be presumed by strict adherence to the list of Provocative Questions enumerated by that agency’s leaders. Rather, selections were based on determination of those areas of investigation that demonstrated the greatest potential for direct and positive implications for radiation oncology. The topics listed do represent a consensus of the TF membership.

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Clinical Translation and Biomarkers

**Current status**

A biomarker can be defined as “a measurable characteristic of a biological system that is indicative of normal function or disease state of the system or its response to an external factor such as a therapeutic intervention.” While in oncology the primary focus of biomarker development has been as a tool for the early detection of cancer, recent developments in laboratory technologies has led to an expansion in research in biomarker science into other areas. In each case, the goal is to identify proteins, the measurement of which provide useful information about an individual patient’s disease status or risk of disease. Despite this effort, few biomarkers have entered widespread clinical use in oncology.

The science of biomarker development is a multistage process, similar to that in place for drug development, and can be divided into 5 phases, termed a prospective specimen collection, retrospective blinded evaluation (PROBE) design. Pepe and Prensner, review this process in detail. Phase 1 involves preclinical exploratory studies, for example, comparing tumor tissue to non-tumor tissue, to identify potentially useful candidates. In this phase, it is important to determine how well the marker distinguishes between cases vs. controls, using statistical techniques such as sensitivity, specificity or receiver operating characteristic (ROC) curves (plots of true positive rate vs. false positive rate). In Phase 2, a clinical assay is developed than can be used to distinguish the disease state from normal on easily obtainable specimens, e.g., blood, urine, etc. During this phase, the reproducibility of the assay on independent cohorts should be assessed, as should its degree of difficulty to perform and its cost. Sensitivity, specificity or ROC curves should be reestablished comparing measurements made in phase 1 vs. those made in phase 2, since markers found to be useful with tissue specimens might not be useful on other more easily obtainable samples. In addition, determining the effect of demographic factors, such as sex and race, and ensuring that the test is useful on archival samples should occur in this phase. Phase 3 consists of retrospective analysis of clinical data sets. For example, clinical specimens collected from patients with tumors resistant to radiation might be compared to those with tumors sensitive to radiation to detect the capacity of the biomarker to distinguish between these 2 populations. In this phase, criteria for defining a positive test are determined, and potential markers are compared to try to identify the most robust. Phase 4 involves the conduct of prospective screening studies. Important considerations include proper matching of control and experimental cohorts, defining proper clinical endpoints, cost and ease of performing the assay, and whether or not the assay ultimately improve stratification between cases and controls. The final phase (Phase 5) involves large-scale population studies. In this phase, the biomarker is validated in a broad setting, determining its reproducibility across different patient groups, establishing whether it improves patient management, and whether it is cost effective.

The number of articles describing the identification of promising biomarkers numbers in the tens of thousands, but most reports are phase 1 studies. Thus, the vast majority of potential biomarkers have not been validated or subjected to the rigor of subsequent phases of analysis necessary for widespread adaptation into the clinic. In order for a biomarker to progress through the next phases of evaluation, researchers must have access to adequate sample sizes reflecting appropriate patient populations. This is especially true in Phases 4 and 5, in which required sample sizes may be in the thousands. Consider the case of prostate specific antigen, in which the large-scale trials evaluating the utility of this marker as a screening test for prostate cancer enrolled tens of thousands of patients. Recently, reporting recommendations for tumor marker prognostic studies have also been published in an attempt to further standardize biomarker science.

Currently, researchers have moved beyond cell-based assays to various “omics” based studies to identify candidate (Continued on next page)
biomarkers for testing in Phase 2-5 studies. The potential applicability of each of these new technologies to clinical radiation oncology will be discussed below.

**Future Potential**

Currently, there are no biomarkers that are in routine clinical use in radiation oncology, unless one considers the dose volume histogram to be a potential marker of normal tissue complication risk in certain circumstances. At this time, there are three areas of study most relevant to the radiation oncology community: tumor radioresistance, normal tissue radiosensitivity, and radiation biosimetry (the latter being most applicable for matters of concern for national security rather than the treatment of cancer patients, and will not be discussed herein).

**Tumor Radioresistance**

As the response of both normal and tumor tissues to ionizing radiation becomes better understood, much work has focused on DNA damage response pathways as potential targets to improve tumor radiosensitivity. An example of one such pathway is the Epidermal Growth Factor Receptor (EGFR) signal transduction pathway, which is a marker of cancer cell proliferation, has been associated with radioresistance and has been successfully targeted for therapeutic intervention in the clinic. Unfortunately, there is a paucity of other similar markers of radioresistance in routine clinical use, despite the fact that more than 50 such proteins have been identified that might play a role in radioresistance. Most have not been confirmed or validated in subsequent studies. A number of different proteomic approaches have been utilized to identify potential markers that are differentially expressed between radiosensitive and radioresistance tumors. This approach has been termed comparative proteomics. The most widely used approach has involved the use of mass spectrometry in which proteins are identified through the use of public databases. Newer approaches involve the use of microarray chip-based screening using antibodies to specific proteins. Unlike genome-wide studies, discussed below, there is no single method yet available that can analyze the entire proteome in a single experiment. One problem is that high throughput technologies may be discovering proteins that are stress-associated artifacts, thus careful confirmation of identified candidate proteins will be required. Semiquantitative methods, such as Western blotting, or analysis at the mRNA level looking for differential protein transcription, may be necessary to confirm potential candidate proteins. Also, analysis of tissue specimens is technically difficult, so nearly all candidate proteins have been identified in cell culture experiments only. A major impediment to confirmation and validation is the lack of availability of archival tumor samples in sufficient numbers. Nevertheless, candidate proteins are beginning to emerge. For example, Feng et al identified 3 proteins associated with radiation resistance in a nasopharyngeal carcinoma cell line (SERPINB5, SFN and SOD2), which were validated in a series of 90 archival patient biopsy specimens obtained pretreatment. Decreased expression of SERPINB5 (promotes apoptosis) and SFN (cell cycle arrest) and increased expression of SOD2 (free radical scavenger) were associated with radioresistance. A panel of these proteins predicted radioresistance with a high degree of sensitivity and specificity. While these data require confirmation, future studies on samples collected in large centralized tumor banks should enable the identification of protein expression patterns associated with both radioresistance and radiosensitivity. Since drugs targeting some of these candidate proteins, e.g., PARP1, already exist, the confirmation and validation of these candidate proteins should lead directly to clinical trials of rationally designed radiosensitizers.

**Normal Tissue Toxicity**

To date, the greatest effort has been focused on identifying biomarkers to predict normal tissue toxicity from radiation therapy, especially late toxicity, which is the major factor limiting the amount of radiation that can be delivered to a tumor in most circumstances. Efforts to identify biomarkers predictive of normal tissue toxicity date back nearly 20 years, but as yet there are none identified that have entered widespread clinical practice. Bentzen et al have proposed distinguishing between 3 different classes of biomarkers of normal tissue research: predictive factors, response markers and surrogate endpoints. Predictive factors are biological or clinical factors observable at baseline (i.e., pre-treatment) that are statistically associated with the probability of a given outcome of a specific treatment in an individual. For example, if an elevation in marker X before treatment correlated with the risk of late lung injury, this would be a predictive marker. Response markers are therapy-related changes in biomarkers that are mechanistically related to treatment outcome at the individual patient level. An example of this type of marker would be marker Y that increased during treatment in patients who went on to develop rectal bleeding, but not in those who did not experience this toxicity. Surrogate endpoints are measurable biological effects that can be used as an early indicator of the effect of therapy on a given clinical endpoint in a population of patients, such as an acute toxicity that had a high probability of resulting in a late toxicity.

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Owing to the recognition that late radiation toxicity appears to have a genetic component, the field of radiogenomics has emerged to study genetic variants associated with the development of late radiation toxicity. Until recently, most studies have focused on the identification of one or a few genetic variants related to the expression of proteins linked to the development of abnormal wound healing, inflammation or radiation injury specifically. The primary approach taken to study this phenomenon in the near future will be genome-wide association studies (GWAS). Designed to identify single nucleotide polymorphisms (SNPs) associated with this endpoint. A SNP is a point mutation, i.e., an alteration in a single nucleotide, that is present in at least 1% of the population. Since humans have paired chromosomes, one may be homozygous for either the common allele or the minor allele, or be heterozygous (have one copy of each allele). The reader is referred to West et al. for a glossary of genomic terms. Because of the need for very large sample sizes to perform these analyses, an international consortium, the Radiogenomics Consortium, has recently been established to focus on these investigations. The initial goals of the consortium are to perform meta-analysis of existing data to confirm or refute previously studied candidate SNPs thought to be involved in radiation toxicity. However, the validity of GWAS studies in identifying genetic variations responsible for human disease is far from universally accepted. The prevailing model driving the search for disease associated genes has been the common disease-common variants model, which postulates that common diseases arise from the additive or multiplicative effects of combinations of common variants on different alleles, each of which confers only a small degree of risk and so multiple variants must be present in an individual to result in the condition. However, GWAS studies have identified many common variants statistically correlated with various conditions that have no established biological function and may even be located in non-coding regions of the genome. McClellan and King argue that such findings are false positive results, and that rare mutations of severe effect are responsible for a significant portion of complex human diseases. More work needs to be done to settle this debate. However, newer sequencing technologies, termed next generation or NexGen sequencing, may permit better identification of disease causing variants based on genome wide sequencing strategies. The challenge using this approach, as with GWAS, will be determining which of many potentially important mutations play a role in an individual’s condition. Thus, access to strong bioinformatics capabilities will play an increasingly important role as more and more information is generated by newer technologies.

The initial goals of the consortium are to perform meta-analysis of existing data to confirm or refute previously studied candidate SNPs thought to be involved in radiation toxicity.

In addition, genetic studies fail to take into account changes in gene expression that cannot be attributed to any changes in the primary DNA sequence. Such changes are heritable, and are referred to as epigenetic changes. The best known epigenetic mechanisms include DNA methylation and posttranslational modifications of histone proteins. Recently, Kuhmann et al demonstrated evidence of DNA methylation changes in MCF7 breast cancer cell lines after fractionated radiation that led to alterations in radiation sensitivity through changes in apoptotic signaling. More work in this area will lead to a better understanding of the impact of epigenetic changes on both normal tissue and tumor sensitivity. Modifiers of histone acetylation are available and are in clinical trials as tumor radiosensitizers, but their role in normal tissue response remains to be determined. Finally, the emerging field of radiomics appears to offer great potential for studying signal transduction pathways in cancer. This field involves the extraction and analysis of large amounts of quantitative imaging data using high-throughput analysis of images obtained with a variety of imaging modalities, including CT, PET or MRI. Opportunities to apply this technology to both tumor radioresistance and normal tissue toxicity risk should be developed. As with genomics, the ability to quickly process large amounts of data rapidly will be essential.

**Needs Within The Profession And Obstacles To Progress**

The establishment of clinically useful biomarkers for both tumor radiosensitivity and normal tissue toxicity risk will be essential as the field of radiation oncology enters the era of personalized medicine. The future study of biomarkers and their ultimate translation into the clinic will depend on several factors. First and foremost, the availability of a large number of appropriate samples on which to conduct studies of tumor radioresistance and normal tissue toxicity risk will be essential, and these sample banks should be developed over the next 5 years. Not only will tumor tissue be necessary for early phase biomarker studies, but also more easily obtainable specimens of bodily fluids, such as blood and
urine, will be essential for subsequent phases of analysis. This will require the establishment of either centralized biorepositories, or multiple smaller biorepositories at individual institutions collected and maintained using standard operating procedures and with centralized oversight.

It will be important to ensure concurrent collection of clinical and demographic information, and that there is adequate representation of different populations within the US. For example, given the evidence of genetic differences in normal tissue radiosensitivity, one cannot assume that the prevalence of SNPs predisposing to normal tissue radiosensitivity would be the same amongst all ethnic groups. In addition, wider access to high level bioinformatics capabilities will be essential to process the enormous amount of information that will be generated on each patient. Beyond the earliest phases of discovery, multi-institutional trials will be required to translate candidate biomarkers into the clinic. This will require cooperative group-level protocol and data management capabilities. Since these studies are not “therapeutic trials” per se, sources of funding will need to be identified to support this work, especially collaboration with industry partners with interest in biomarker development and testing. It will also be important to have scientific advisory panels convened at regular intervals to evaluate the merits of proposed studies utilizing these resources, since the availability of these samples will be finite, and only the best candidate biomarkers should move on to large scale clinical evaluations.

The application of “omics” technologies to radiation oncology is in its infancy. In addition, the technologies are rapidly evolving, and although the costs are decreasing, they remain relatively high. Early phase studies (discovery) remain the province of individual investigators, but later phase studies will require resources beyond the pale of even the largest institutions. Thus, the first priority would seem to be to establish the infrastructure necessary to conduct the higher phase studies to validate the most promising markers, and to develop partnerships with investigators and industrial partners already engaged in this type of work. For example, collaboration and consultation with the NCIs Early Detection Research Network (EDRN), originally established to develop cancer-screening biomarkers and move them through the first 3 phases of testing, should help to guide the development of these studies as applied to radiation oncology.

CHAPTER REFERENCES


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Current status

Cells respond to changes in their extracellular and intracellular environment by activating signal transduction pathways. Signal recognition is through a wide variety of sensing mechanisms, often involving trans-membrane receptors. A coordinated cascade of sequential protein-protein interactions involving conformational changes, post-translational modifications (PTMs), and enzyme activation transduce the signal and elicit a response. The endpoint may be phenotypic changes but often result in temporally controlled activation of numerous genes, via both transcriptional and post-transcriptional mechanisms, encoding a broad range of regulatory and effector proteins that alter cell metabolism, cell cycle, function, or fate. These responses are cell type specific with sensors being intimately linked to lineage developmental pathways at one level and at another to regulatory chromatin barriers that must be eliminated to allow transcription. Primary phase genes provide a very responsive system being activated rapidly without de novo protein synthesis, while secondary genetic programs that are initiated more slowly function for long term cellular reprogramming are under greater regulatory control. Signaling mechanisms are obviously multiple and complex, but converge on a number of canonical pathways that are essential in determining cell fate in a given microenvironment and are universal primordial means of cell regulation that are essential for all multicellular and unicellular life.

Second messengers, such as cAMP, cGMP, inositol triphosphate (IP3), Ca2+, nitric oxide (NO), and/or reactive oxygen species (ROS) generally increase rapidly during cell stimulation and amplify and may mediate responses. Several canonical pathways may be activated simultaneously even by a specific ligand-receptor interaction and cross-talk can provide alternative signaling routes. This can be a problem when relay molecules are targeted for inhibition as alternative pathways can bypass the block.

The best-known PTM involves phosphorylation or dephosphorylation of Ser/Thr or Tyr residues on proteins by activated kinases or phosphatases, respectively. Less information is available on the roles of other PTMs such as acetylation, ubiquitination, glycosylation, nitrosylation, oxidation, lipidation, and methylation but in all cases the particular protein residues or peptide links that are modified provide a footprint of the signal transduction pathways that are modulated. This footprint is best assessed by functional proteomics.

Ionizing radiation (IR) can activate multiple signal transduction pathways, depending on dose, dose rate, radiation quality, cell type and many other variables. Indeed, one reason conventionally fractionated radiation doses are superior to high doses in exploiting differences between tissues may be that they allow a greater role for signal pathway activation. The “signal” from IR is the energy deposited in ionization events and primary free radical production, which is amplified by ROS generated indirectly from many intracellular sources, as well as by nitric oxide production, ion fluxes, and other second messengers. Since ROS are critical second messengers that are generated in many if not all signal transduction pathways, signal transduction is a major point of confluence between physical energy deposition by IR and biology. A sensitive and relevant target for ROS is the thiol proteome; proteins with constitutive cysteine residues. The fact that many transcription factors such as NF-κB, Nrf-2, AP-1, PPAR-γ, p53, Sp1, STAT3, and HIF-1 are part of the thiol proteome speaks to the importance of redox in signal transduction and the control exerted by pro- and anti-oxidants, IR being largely pro-oxidant. Phosphatases are particularly redox-sensitive. Their inactivation is probably involved in the activation of EGFR-MAP kinase pathways within minutes of IR exposure. Other possible membrane-linked targets include purinergic P2X receptors involved in ligand-gated ion channels and the sphingomyelinase-ceramide pathway. However, the best-documented IR-induced pathway mediated is the DNA damage response (DDR) that uses primarily ATM and ATR kinases as signal transducers to trigger p53 and p21 activation with apoptosis and cell cycle arrest as possible radiation-induced outcomes.1

A major aspect of intrinsic cellular response to IR depends on the signals that are transduced and how they integrate within what is a complex intracellular milieu. Many variables such as the basal expression levels of sensors, second messengers, redox status, the existing metabolic and genetic programs, and microenvironmental signals will have an

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impact on the IR response. In cancer radiation therapy (RT), cancer-associated mutations will play a major role as these generally constitutively activate signal transduction pathways, and impact tumor radiosensitivity. Understanding the footprint of IR within these settings is therefore essential for understanding why some cancers respond to IR and others do not, as well as for identifying possible biological targets for improved radiotherapeutic intervention.

Current resources
The primary importance of signal transduction pathways in radiation oncology is that they can be manipulated to alter intrinsic cellular radiosensitivity. There are numerous examples demonstrating this principle using cytokines or growth factors, drug or antibody inhibitors, or genetic manipulation of pathways. For tumors, it has been known since 1985 that RAS oncogene expression could enhance intrinsic cellular radioresistance. Indeed most, though not all, oncogenic mutations negatively impact tumor cell response to IR. The effects are through constitutive activation of signal transduction pathways in cancer cells that select for cells with mutations that steer them away from apoptosis and towards cell proliferation and survival. As a result, increased radioresistance has been ascribed variably to activation of canonical EGFR-P13K-AKT, Ras/Raf/MEK/ERK, ATM-NF-κB, or Jak-STAT pathways or mutations in the ATM-p53 pathway. Hundreds of drugs that are relatively specific for target molecules within signal transduction pathways are in development and about 50 are in clinical trials. The radiation oncology field has lagged others when it comes to exploiting the potential of these drugs, although the Phase III trial targeting EGFR with Cetuximab in head and neck cancer patients receiving RT increased overall survival and local tumor control. Also, a Phase II trial targeting VEGF with Bevacizumab along with 5-fluorouracil and RT in colorectal cancer showed efficacy.

In reality, perhaps the radiation oncology field is not so far behind others. While Gleevec provided the paradigm for targeted therapies, the benefits from targeting pathways in most solid tumors, with or without chemotherapy, have been marginal, coming with increased toxicity and considerable financial cost, indicating that there is much still to be learned about how best to use molecular targeted agents. Many suggestions have been proposed for the relatively disappointing outcomes. The most favored is tumor heterogeneity. A poster-child is the finding by some (but not all!) groups that patients with colorectal cancer whose tumors harbor KRAS mutations do worse than those with wild-type tumors if Cetuximab is included in their therapy, suggesting that upstream targeting is ineffective in the presence of a downstream mutation. Since tumors from patients with advanced tumors may have hundreds of mutations, this would not be expected to be a sole solution. This is indicated by findings in some trials that patients receiving EGFR-targeted drugs unexpectedly did worse than normal. In patients receiving pre-operative RT and chemotherapy for rectal cancer the addition of Cetuximab was a negative indication. In this trial, the influence KRAS was not seen and the response to IR, not chemotherapy, was negatively impacted (pers. comm.).

Understanding the signaling pathways involved will be critical if molecular targeting is to be combined with cancer RT. However, it should also be remembered that molecular targeting is rarely cytotoxic and unlikely to be effective alone or in combination with minimally cytotoxic agents. The probable advantages of combining them with a known powerful cytotoxic agent like RT would seem obvious in the appropriate setting. Furthermore, it is clear that the current efforts to mitigate the consequences of large-scale release of nuclear or radiological material as a result of deliberate attack or natural disaster is yielding products that modify normal tissue responses. Given that tumors and normal tissues have different response pathways, dual-acting agents should be available that may be of considerable future clinical value in RT for cancer.

Future potential
An understanding of how cells transmit radiation signals so as to modify their own behavior and that of others in local and distant sites is arguably the greatest gift that radiobiologists could give to clinical radiation oncology at this point in time. As a signal, IR triggers canonical pathways that go back to the beginning of life. However, its functional proteomic footprint will be unique as its targets are distributed throughout the cell, rather than being for example a single type of membrane receptor. At clinically relevant doses, the DDR is likely to be an imposing feature but the response is by the whole system and will involve many elemental pathways. More detailed profiling of the radiation footprint and how it is interpreted in the context of different genetic backgrounds, different cancer mutations, and different microenvironements and how this influences cellular fates and reprograms cells and tissues is essential if radiation treatment is to be personalized. This will take much effort, but the possible rewards are great.

One facet of the multiple outcomes of signal transduction is extracellular signaling. This also will influence the outcome of RT. In the radiation field, these have been called “bystander” or “non-targeted” effects though most biologists would view them as intercellular signaling mechanisms. Irradiated tissues and cancerous tissues send “danger” signals that act locally, regionally, and distantly. These are critical.

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in establishing the tumor-host relationship and the microenvironment in which therapies have to function. The implication is that all cancer and all cancer therapies have a systemic component, even if IMRT is delivered to a non-metastatic tumor. An integral part of cancer therapy should be the rebalancing of the processes that were disturbed by the cancer and by the therapies that were employed. Profiling the host response to cancer and therapy will be of relevance to therapeutic outcome in the short term, but since imbalances continue in a chronic form, perhaps for the life of the patient, profiling the pathways activated late after RT and rebalancing these should be considered part of cancer treatment.

The potential impacts of profiling signal transduction pathways therefore lie in several aspects of clinical radiation oncology. The first is in understanding the patient’s cancer and the signaling pathways that are dysregulated in order to personalize treatment. Radiation Oncology treatment schedules are for the most part predicated on normal tissue tolerance to IR. If treatment is to be personalized, a broader attitude that recognizes the heterogeneity of tumors and their response to therapy will have to be adopted. Second, if molecular targeting is to be optimally integrated with RT, the combinational effects have to be studied within a clinically relevant setting and meaningful molecular endpoints established. Because IR has a unique footprint, molecular radiotherapeutic drugs should be identified rather than assuming that what works with chemotherapy will work with RT. Both cancer and local and systemic host responses will need to be included in this equation. Thirdly, the long-term imbalances caused by the cancer and the therapies employed will have to be addressed. Profiling the signal transduction pathways that are activated late after treatment will help guide therapeutic intervention. Study of clinical late effects should be regarded as part of cancer treatment.

**Needs within the profession and potential obstacles**

Classical radiobiology has its roots in target theory and random deposition of energy that stresses the importance of cell death, largely by mitotic catastrophe. Because free radicals, in particular ROS, were thought to be the mediators of radiation cytotoxicity, free radical scavenging or donation were considered the optimal way of modifying radiation responses, with tumor hypoxia being the perfect example of induced radioresistance. Differences in “repair” mechanisms between late and acute responding tissues were proposed to be why fractionated 2 Gy doses were superior to high doses in RT, without “repair” ever being fully explained. This paradigm has to change with signal transduction being placed at the heart of intrinsic radiation responsiveness and hypoxia being seen as a “signal”. The field of DNA damage and repair, which is actually quite well represented in Radiation Oncology departments in the USA, has traditionally developed as a field of study in itself with little cross-over into the clinic or into broader radiobiological topics. This needs to change, particularly since many molecular targeted agents affect DNA repair. It is rather ironic that the first description of the effect of RAS on radioresistance pointed out its dose rate dependency indicating an effect on DNA repair pathways, but the relationship between EGFR signaling pathways and repair of DNA damage has only recently come to the forefront. After several previous models were found lacking, the linear quadratic model became the standard method to describe the effects of changing fraction size while the 4 R’s became the variables that impacted fractionated radiation delivery. These strong, clinically relevant, quantitative concepts became the biology accepted by the profession, while all aspects of diversity and its underlying mechanisms such as signal transduction and genetics were largely ignored. As a result, the field was poorly prepared for the biological revolution, systems biology, and for personalized medicine.

The time for personalized medicine is clearly now. For molecular targeting to become part of radiation oncology, the uniqueness of the IR footprint on signal transduction pathways needs to be established in individual tumor and normal cell and tissue systems and its impact on molecular targeting approaches established in vivo.

For molecular targeting to become part of radiation oncology, the uniqueness of the IR footprint on signal transduction pathways needs to be established in individual tumor and normal cell and tissue systems and its impact on molecular targeting approaches established in vivo. (Continued on next page)
CHAPTER REFERENCES


Tumor Microenvironment and Hypoxia

Current status

The cancer cell-centric view of solid tumors that dominated laboratory and clinical investigations for the past several decades has evolved recently with a growing recognition that the tumor microenvironment and its components play a critical role in disease manifestation. A comprehensive review of the current status of research focused on tumor microenvironment and hypoxia is clearly beyond the scope of this commentary and several exceptional reviews are available (e.g.1). However, conceptual organization of this research may aid in mapping out future directions.

Roughly, this broad-ranging topic can be divided into two critical, interrelated topics relevant to the radiation oncology/biology community. First, considerable progress has been made on the basic biology of stromal-cancer cell interactions and their impact on the malignant disease spectrum (carcinogenesis, progression, metastasis). Second, and far less developed, is an understanding of how these interactions may be exploited for therapeutic gain.

Perhaps the best-developed area of tumor microenvironment research is the role of vascular (and perivascular) cells, and angiogenesis in particular, in tumor development, progression and metastasis. Neovascularization is now well accepted as a critical, central feature in the transition of tumors from small, clinically irrelevant cancer cell masses to clinically relevant disease.2 This process is essential for delivery of needed blood-borne nutrients and oxygen to burgeoning malignancies. As a result of the so-called “angiogenic switch”, cancer cells are spared death secondary to metabolic and hypoxic stress. However, vascular (and perivascular) cells have now been shown to play additional roles in malignant disease. The abnormal structure and function characteristic of solid tumor vascular beds contributes to both chronic and acute hypoxia that may promote a more invasive malignant phenotype.3 Recently, intriguing pro-proliferative cross-talk between vascular (and perivascular) cells and cancer cells has been proposed. Although long recognized that cancer cells promote angiogenesis through production of pro-angiogenic factors, emerging evidence suggests that in some situations vascular and perivascular cells produce factors, termed angiocrine factors, that promote cancer cell proliferation and invasion.4

Stromal contributions to malignant disease extend beyond vascular cells. Infiltrating immune/blood cells, cancer-associated fibroblasts and the extracellular matrix itself have been implicated in an array of cancer-promoting activities. For example, increased numbers of CD4+ CD25+ T-regulatory cells have been identified in a variety of human tumors and are known to inhibit antitumor immune responses.5 Tumor-associated macrophages and other infiltrating leukocytes produce growth factors and chemokines that promote cancer cell proliferation as well as proteases that contribute to matrix remodeling and metastasis.6 More subtle inflammatory effects on tumor progression have also been suggested by observations that anti-inflammatory medications can restore oncogene-induced senescence in some murine models.7 Cancer-associated fibroblasts have been shown to pathologically modify the extracellular matrix, secrete mitogenic factors, promote epithelial-to-mesenchymal transition and unfavorably modulate tumor cell metabolism.8, 9 The cancer-associated extracellular matrix has been implicated in the malignant phenotype, as a storehouse of mitogens and as a regulator of cancer cell metabolism.10-12 Clearly, the past decade has seen enormous progress in dissecting the multitude of stromal-cancer cell interactions that bear on disease manifestation.

In sharp contrast, relatively little progress has been made in exploiting this rich body of literature for improving the efficacy of radiotherapy. Although antiangiogenics, and more recently, immune modulators have proven efficacy as monotherapy or in combination with cytotoxic chemotherapy, the impact of tumor microenvironment-directed therapies on outcomes of radiation-centric regimens as been modest. An instructive example is the elegant work of Christopher Willett and Rakesh Jain on the use of the antiangiogenic, bevacizumab, in locally advanced rectal cancer.13, 14 Despite compelling evidence that bevacizumab exerted the hypothesized primary tumor vascular normalization, the impact of this agent on local control and pathological responses when combined with conventional chemoradiation appears limited. Moreover, conflicting data on the ability of antiangiogenics to improve vascular function and thus cytotoxin and radiosensitizing oxygen delivery remains a barrier to optimal incorporation into radiation regimens.15

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**Future potential**

As discussed above, discovery centered on the basic biology of tumor microenvironment influence on the cancer spectrum has far outpaced our ability as radiation oncologists to exploit this new knowledge for therapeutic gain. Inarguably, there remains a need to further refine our understanding of the basic biology of stromal-cancer cell interactions. However, from the perspective of the radiation oncologist, the greatest potential advances lie in the exploitation of existing and emerging basic science to improve the efficacy of radiation therapy. Examples of the painstaking process of genuinely translational radiation research are relatively rare but may serve to simultaneously highlight both the challenges and potential of these efforts.

One prominent example of bench-to-bedside translational radiation research exploiting basic tumor microenvironment insights is the collaborative efforts of Jain, Willett and colleagues. This multidisciplinary team started with a hypothesis derived from years of preclinical investigation into tumor-associated vasculature and the impact of antiangiogenic therapy on tumor biology. Specifically, this team endeavored to examine the “vascular normalization hypothesis” that posits judicious use of antiangiogenic agents will enhance the function of the abnormal tumor vasculature thereby improving cytotoxic therapy delivery and radiosensitivity via improved oxygenation. Informatively testing this hypothesis in a clinical setting presented an array of challenges. What metrics could be realistically employed to assess tumor vasculature in a dynamic way? What patient cohort could be ethically examined? What clinical endpoints should be examined? Eventually, the design of the clinical trial was an innovative balance between the desire to extend fundamental stromal biology insights to the human setting and offer patients a promising new therapeutic regimen. Consenting patients with locally advanced rectal cancer were treated with induction bevacizumab followed by conventional chemoradiation plus bevacizumab. Importantly, patients were also subjected to repeat sigmoidoscopic examination and biopsy following induction bevacizumab permitting assessment of tumor regression and potential biomarkers of vascular normalization including microvascular density, interstitial fluid pressure and perivascular cell coverage. Functional computed tomography and 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) were integrated into the trial to provide non-invasive insights into tumor blood perfusion/volume and metabolism, respectively. Circulatory biomarkers were also explored. This correlative study-rich trial provided novel, hard-earned insights into the effects of bevacizumab on tumor vascular biology that would have been discernable from a more classic, empiric clinical trial.13 In addition, this team continued to accrue and examine patients and deserves considerable credit for both (a) attempting to evaluate the impact of induction and concurrent bevacizumab on conventional chemoradiation outcomes and (b) exploit lessons learned in refining additional trials of antiangiogenic therapy.14 As is the norm, this trial answered several questions (and largely supported the vascular normalization hypothesis) but simultaneously raised several others. Does vascular normalization enhance radiosensitivity? This trial did not examine tissue oxygenation and clinical results suggest the addition of bevacizumab did not substantially increase the rate of pathologic complete response or local control compared to standard therapy. Does vascular normalization enhance chemosensitivity? The trial yielded favorable disease free and overall survival rates compared to historical controls. Although this comparison is fraught with potential biases (including different rates of adjuvant chemotherapy use), it raises the possibility that, as in the metastatic setting, the addition of bevacizumab improves outcome in combination with cytotoxic chemotherapy. If a vascular normalization window exists that can be exploited with radiation and/or chemotherapy, how should it be identified and how long does it persist? Answers to these questions are critical in designing optimal sequences of intervention. This ongoing experience highlights both the potential and the challenges of multidisciplinary translational trials focused on exploitation of stromal biology in radiation regimens.

Similarly, the team of Silvia Formenti, Sandra Demaria and colleagues have dedicated considerable effort to exploiting knowledge of the tumor microenvironment to enhance radiation efficacy. In short, this team endeavors to transform solid tumors into effective, in situ, individualized vaccines with combinations of radiation and immunomodulators.

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In 2004, this team reported that an abscopal effect on unirradiated tumors could be induced by radiation of an anatomically distinct tumor in the presence of Flt3-ligand used to expand the dendritic cell compartment and promote cytolytic T-cell responses. Since that time, this team has steadily advanced this notion to clinical practice culminating, recently, in the initiation of a proof-of-principle clinical trial designed to detect and characterize the abscopal effect in humans. Specifically, patients with multiple measurable lesions and stable/progressive disease on systemic therapy were treated with two weeks of fractionated radiotherapy directed at a single lesion. After a week of radiation therapy, patients were initiated on daily GM-CSF to enhance tumor-specific immune responses. Final results are awaited, however, an abscopal effect resulting in a partial response of an unirradiated lesion was detected in approximately one third of patients. Abscopal responses were even more common when examined by a reduction in FDG avidity using PET. These observations and additional evidence provided by this team raise the important and intriguing possibility that radiation therapy may find a place in addressing pre-existing, distant malignant disease - a role rarely envisioned for this “local” therapy. From these and other similar translational successes emerge some common themes essential for maximizing the clinical potential of tumor microenvironment studies. First, successful translational studies are borne of a basic biological hypothesis supported by ample pre-clinical evidence. Second, the teams guiding translational studies are composed of, or are at least led by, stable, committed investigators that maintain collaborations for many years. Third, through force of will or institutional commitment, a multidisciplinary infrastructure must exist to support the spectrum ranging from preclinical observation to clinical trial. Simply put, teams must include tightly integrated, basic, translational and clinical investigators and often must have representation from multiple fields within these three large categories. Consider the rectal cancer studies discussed above. This team was variably composed of basic scientists, radiation oncologists, medical oncologists, radiologists with an array of expertise (CT, PET), gastroenterologists and pathologists. These themes serve to highlight the needs and potential obstacles to progress in exploiting the tumor microenvironment for therapeutic gain.

**Needs within the profession**

Successful translational of tumor microenvironment insights into clinical radiation oncology, like all science, requires investigators benefit from four critical elements: space, time, money and the right environment. The former and latter are less daunting concerns. Research space supporting translational science is of course critical and precious, however, many of the key elements preexist in medical centers and support routine clinical care. Consider the structural/environmental requirements of the rectal cancer trial discussed above. The imaging, medical oncology, radiation oncology, pathology and gastroenterology infrastructure required by the correlative science in this trial were well established and staffed by top-notch clinicians. In this instance, the formidable challenge was to bring these disparate resources seamlessly to bear to address a key tumor microenvironment question. The additional key environmental factor is talented investigators dedicated to collaborative, translational medicine. In this regard, our field actually suffers an embarrassment of riches. Consider the sea change in radiation oncology over the past two decades. There has been a striking influx of physician-scientist applicants to radiation oncology residencies. The Holman Research Pathway provides radiation oncology residents a mechanism to augment prior research training and prepare them for a career in investigational medicine. And yet, the availability of research-centric, radiation oncology positions has not kept pace with supply. The observation that graduating residents with credentials and a dedication to basic, translational or clinical science are having difficulties finding research positions merits study as the causes are likely multifactorial and the consequences may be far-reaching. Medical students are highly responsive to medical specialty trends and the inability of radiation oncology residents to find (and maintain) research-oriented positions may drive talented, prospective radiation oncologists to fields where research-oriented positions are more abundant.

The remaining two elements – time and money – are interrelated and exceedingly problematic in the current fiscal environment. There is a significant opportunity cost associated with a clinician substituting well-reimbursed clinical effort for increasingly poorly funded research. In radiation oncology, there is evidence that early investment in junior research faculty pays dividends in terms of additional grant support and publication productivity but this trend must be intensified to address the shortfall created by NIH and other organizations. Additionally, hard choices in terms of funding priorities must be made; not all meritorious research will be funded. An open, spirited debate about where tax and philanthropic dollars are best dedicated will be essential for shaping the field of radiation biology in the future. Finally, solutions to the challenges of limited time and financial support must be aggressively promulgated to trainees lest we risk “a lost generation” of radiation biologists.

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CHAPTER REFERENCES


**Current status**

Radiation sensitizers and protectors are generally described as agents that can modify the biologic effects of ionizing radiation. While non-chemical modalities have been studied as radiation sensitizers and protectors, most research in this area has focused on chemical modifiers. Traditional radiation sensitizers and protectors are defined as inactive agents that increase or decrease the effectiveness of radiation, respectively. Importantly, these terms are context dependent, and the specific end-point of effectiveness may vary in different biological systems. Most chemicals studied for their putative sensitizing properties are biologically active and exhibit spatial cooperativity rather than traditional radiosensitization.¹

Many preclinical studies have been performed to assess the radiation modifying effects of different types of chemicals (cytotoxic, biologic, molecularly targeted). The most common research approach to study radiation sensitizers involves exposure of cancer cell lines to ionizing radiation in the presence or absence of a chemical. The end-point of this experiment is clonogenic cell survival. Alternatively, cancer cell lines are used to generate a tumor in an animal. This tumor is irradiated in the presence of absence of a chemical. The endpoint of this experiment is tumor control or regrowth delay. The relative advantages and disadvantages of these models have recently been reviewed by Kahn and colleagues.² Studies of radiation protectors typically involve more complex sequelae and focus on organ system effects in an animal.³ With the expanding number of chemicals in pharmaceutical development, it is likely that studies of this nature will continue.

Despite the number of preclinical studies suggesting potent radiation sensitizing or protecting effects of various chemicals, few have been tested in clinical studies. Glass and colleagues estimated that fewer than 10% of phase I cancer clinical trials between 2001-2009 incorporated a chemical therapy with radiation therapy.⁴ A query of the National Cancer Institute’s Clinical Trials website (www.cancer.gov) revealed 110 active studies of “radiosensitizers” or “radioprotectors” (Figure 1); the majority (88%) of trials focused on radiosensitizers, with most in phase II of study. Few studies have attempted to incorporate biologic endpoints that confirm target activation or inhibition, and therefore improved mechanistic understanding in humans is typically not gained in clinical trials.

**Current resources**

Several resources exist to support research of radiation sensitizers and protectors. The Cancer Therapy Evaluation Program (CTEP) and Radiation Research Program (RRP) of the National Cancer Institute (NCI) represent sources of federal expertise and support. The pharmaceutical industry is a potential source of support for studies of newly developed drugs that can be combined with radiation as putative radiation sensitizers or protectors.

**Future potential**

Several potential areas for future research of radiation sensitizers and protectors exist. Most radiosensitizers have been characterized because of biologic activity as single agents. For example, 5-fluorouracil is used in conjunction with radiation therapy for gastrointestinal malignancies and is active as a single agent in these cancers. For this reason, most clinically employed chemicals used in conjunction with radiation therapy that exhibit spatial cooperation rather than traditional radiosensitization.

Efforts directed to discover traditional radiation sensitizers and protectors that are not independently active have been enhanced through high-throughput screening (HTS) techniques. These experimental systems allow for large-scale identification of biologic targets or drugs that may modulate the response to radiation, but may not otherwise have been suspected to be effect modifiers. For example, a HTS screen of 3600 chemicals with known biologic activity identified 18 chemicals with putative radiation protective properties, including 2 commonly used classes of bacterial antibiotics.⁵ Another HTS screen of 6880 druggable or protein kinase genes revealed uroporphyrinogen decarboxylase as an important mediator of radiation sensitivity.⁶ Discoveries of previously unrecognized modulators of the radiation response seem vital to the exploration of traditional radiosensitizers and radioprotectors beyond the scope of typically assessed anticancer drugs.

Another area of potential research is the development of preclinical models to study radiosensitizers and protectors that better replicate the clinical response to radiation therapy in patients. Most studies of radiosensitizers examine only the effect of radiation on cancer cells and model tumor systems. The end-point of these studies almost never incorporates (Continued on next page)
normal cellular or tissue response. To achieve therapeutic gain, radiosensitizers should ideally demonstrate tumor selectivity, in order not to increase the adverse effects of radiation therapy. While some models of normal tissue and organ response to radiation response exist, a previous assessment of these models suggested that more robust systems are desirable. Specificity of the normal organ systems should be considered (ie, for radiosensitizers of prostate cancer, consider normal tissue/organ effects in bladder, rectum, etc).

Another area that would benefit from further research is clinical trial development and participation. Based on the data in figure 1, radiation oncologists appear to be generally reluctant to test radiosensitizers (possibly because of concerns regarding normal tissue toxicity), and reluctant to test radioprotectors (possibly because of concerns about tumor protection and treatment failure). Rational development in appropriate clinical settings (ie, phase 0-1 studies in patients undergoing low to moderate dose radiotherapy) may improve comfort with radiation effect modifiers, and facilitate future clinical design. Other improvements for the development of early phase clinical trials of radiation sensitizers and protectors have been proposed by the Radiation Therapy Oncology Group (RTOG), the NCI and others.

Finally, the reason that radiation oncologists do not use radiation sensitizers and protectors in practice should be studied. Use of the only Federal Drug Administration approved radioprotector, amifostine, is a highly controversial topic. Generally, radiation oncologists have been slow to embrace the use of radiation effect modifiers. The reason for this is unclear. Are radiation oncologists sufficiently trained in the use of radiation sensitizers or protectors? If effective traditional radiation sensitizers were developed, would radiation oncologists prescribe them? Is reluctance to use radiation effect modifiers a hindrance to development of this field? The answer to these and similar questions are as important as the science that underlies the development of radiation sensitizers and protectors.

### Needs within the profession and obstacles to progress

As a profession, the general focus of radiation oncology has been the study and use of radiation therapy as part of the care of the cancer patient. Chemical therapy that modifies the effect of radiation has seemingly not been a major area for study. The profession needs to support the laboratory and translational efforts in this area if meaningful clinical gains are to be made. The most promising laboratory and translational findings need to be moved to the clinic and embraced by radiation oncologists.

Several simultaneous goals should be considered to accomplish the needs of the profession. The development of tools for radiation sensitizer and protector discovery are becoming increasingly available, but should be implemented in radiation biology research within the next 3-5 years. Work of this nature can be costly, and will be an obstacle. Translational studies that confirm biologic activity and lend

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mechanistic insight are needed over the next 5-7 years. The partnership with pharmaceutical industry, as well as CTEP and RRP will be vital to success. Finally, increasing the number of clinical trials that are undertaken based on promising preclinical data is necessary over the next 3-7 years. This may require improvement in the understanding of the mindset of clinical radiation oncologists, and if necessary, a change in the culture of radiation oncology and should be a priority over the next 2-3 years, so that other efforts are not made in vain.

Improving partnership with the pharmaceutical industry will be critical to foster the development of novel radiation sensitizers and protectors. Given the lack of pharmaceutical support for novel chemicals with radiation modifying effects not in development, partnership with the RRP will be necessary. Clinical trial networks and programs through RTOG and ASTRO will be vital to develop better understanding of radiation oncology clinical needs and challenges to successful implementation.

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Genomics and Epigenetics in Radiation Oncology

Current status
The emergence of DNA deep sequencing has elucidated that tumors are comprised of a heterogeneous mix of cells harboring an array of inherited and tumor-acquired mutations, making a patient's tumor unique to that individual.\(^1\) Consistent with these new findings, over the last several years it has been recognized that the highest rates of success in cancer therapy are achieved when particular drugs are given in combination with tumors harboring particular mutations, referred to as targeted therapies.\(^2\) Targeted therapies include small molecules and antibodies that act on particular tumor-acquired mutations. The extension of personalized therapy has not made it to radiation therapy.

Despite significant technological advancements regarding the delivery of radiotherapy for the treatment of cancer,\(^3\) there is still a wide and unpredictable range of patient response in terms of disease control and normal tissue toxicity. These are trade offs, and biological insight into the specific dose required to cure patients with minimal toxicity would be an enormous advance in the treatment of patients with radiation therapy.

The molecular basis leading to differential outcomes is widely unknown, but if this variability could be identified before treatment, patients could be stratified into response groups and therapeutic regimens could be modified according to the individual. Clearly, biomarkers that are tumor specific would likely have limitations in their ability to predict normal tissue toxicity. Currently, there are no companion diagnostics utilized before a patient undergoes radiation therapy. The identification, validation and subsequent addition of biologic diagnostics to guide radiation therapy would be a significant step towards personalized therapy.

Here we will explore the potential for genome profiling and epigenetics as potential sources and paradigms for biomarker discover and validation to help predict clinical outcomes and direct radiation therapy.

Future potential
Genomics review: The primary basis for radiosensitivity or radioresistance genetic markers stems largely from clinical studies of rare genetic disorders such as Ataxia Telyangiectasia and Nijmegen Break Syndrome. Patients with these diseases harbor germ-line mutations in the ATM and NBS1 genes (respectively), which are critical components of the DNA damage response and repair pathway, resulting in extreme radiosensitization and severe normal tissue toxicity in these patients.\(^4\)\(^,\)\(^5\) Subsequent work studying these genes in radiotherapy has shown that canonical open-reading-frame mutations as well as single nucleotide polymorphisms (SNPs) confer radiosensitization. This work has spawned the search for germ-line mutations and SNPs in other genes involved in DNA damage response and repair (TP53, XRCC1, MLH1 and LIG4); as well as genes involved in cell cycle regulation (CDKN1A and CCNE2); oxidative stress (SOD1 and SOD2); and apoptosis (BCL1, BCL2 and ABL).\(^6\)\(^,\)\(^7\)

The use of cDNA and microRNA microarrays and RNA sequencing has revealed that tissues have specific gene expression patterns characterized by mRNA and microRNA expression.\(^8\)\(^,\)\(^9\) Utilizing genome-wide expression analysis several groups have shown that global gene expression is altered in response to UV and ionizing radiation\(^10\) and more recently that microRNA expression is altered in response to radiation.\(^11\) The logical steps forward would be to identify expression patterns consistent with radioresistance, and use these patterns as diagnostics to tailor radiation dose.

Epigenetics review: Over the course of evolution cells have developed elaborate mechanisms to regulate the temporal and spatial expression of genes, which are required to carry out both normal biological processes and allow cells to adapt/respond to environmental cues, stimuli and stressors. The epigenetic regulation of gene expression is a potent cellular mechanism to control gene expression by creating a regulatory checkpoint at the transcriptional (or DNA) level. Epigenetic gene regulation occurs through two primary mechanisms - DNA methylation and histone modifications.\(^12\)

DNA methylation alters gene expression, and approximately 60% of the mammalian genome is methylated in regions containing CpG dinucleotide repeats of at least 200 nucleotides or more and having a GC content of over 50% located primarily in promoter regions. The role of DNA methylation on inactivation of critical genes required for the DNA damage response and repair pathways could lead to enhanced radiation sensitivity. An example is methylation (Continued on next page)
of O6-methylguanine-DNA-methyltransferase (MGMT), which has been found to be associated with improved outcome in glioblastoma and is a prognostic biomarker for sensitivity to alkylation agents.\textsuperscript{13} This indicates that MGMT promoter methylation may hold potential for use as a predictive biomarker for radiosensitivity in glioblastoma, and possibly other cancers.\textsuperscript{14} In another study, promoter methylation of Reprimo (a tumor suppressor) associated with poor response in esophageal cancer patients who underwent uniform chemoradiation.\textsuperscript{15}

Potential: Even though a wealth of genetics/genomics/methylation information exists in the radiobiology field, there has been very little effort to move such signatures into the clinic, even to perform retrospective analyses on prospective trials as validation studies. There is great potential of panels of such markers to be applied clinically to help tailor therapy if they existed, even today.

Needs within the profession
There already exists a wealth of clinical trial samples with matching clinical outcomes and toxicity metrics through cooperative group studies that are amenable for application of biomarkers of tumor and normal tissue radiosensitivity. Results from such studies could quickly lead to biomarker panels that could be applied to help direct radiation therapy treatment doses to increase tumor control and decrease normal tissue toxicity for individual patients. However, there are several significant obstacles to application of such biomarkers. The primary obstacle is clear interest from the physician community or insurance community that would lead to incorporation of such biomarkers into treatment paradigms, and inspire companies to invest in their development. Unfortunately, the incentive structure is not aligned with the goals of personalizing radiation dosing. While there are numerous examples of diagnostics to direct personalized drug therapies, because radiation is a therapy that works on everyone, there has not been an incentive to reimburse by best outcome (i.e. tumor control) and limited toxicity. Instead reimbursement is based on the number of fractions delivered, and the level of complexity of planning. While theoretically increasing the number of fractions using more complex planning could, and has, led to increased tumor control with decreased normal tissue toxicity, the field of radiation oncology is decades behind other medical fields that use biology, not just technology, to personalize medical decision making.

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CURRENT STATUS

It is firmly established that the DNA damage response (DDR) plays an integral part in pathogenesis of cancer as well as response of both normal and malignant tissues to cancer therapeutics.1 Significant benchtop research over the past decades has identified specific DNA repair pathways as they relate to signaling cell cycle checkpoints (collectively referred to as DDR), and how these pathways contribute to maintaining genomic stability in response to DNA damaging agents.2 Traditionally, cancer therapeutics involve “overwhelming” tumor cells, but not normal cells, with DNA damaging agents in the form of combinations of chemotherapeutics and radiation. A more recent focus on “personalized cancer therapy” is now attempting to understand the underlying molecular defects in tumor which could result in more “targeted therapy” for an individual patient. Therefore understanding tumor biology, as it relates to DDR pathway, would be critical in for future protocols using “molecular-targeted radiation therapy”

As information pertaining to the DDR has evolved, there has been an increasing interest in targeting DDR pathway molecules for cancer therapy. This has arisen for several reasons: (1) fundamental differences in DDR may exist between normal vs. pre-malignant/malignant tissues and (2), some cancers have innate and specific defects in DNA repair which can lead to increased sensitivity to DNA damaging agents. In other tumors, cellular resistance may occur due to a compensatory upregulation of DDR pathway genes. In general, tumors manifest an upregulated DDR activity due to rapid cell proliferation and replication stress, which can contribute to genomic instability and if harnessed, leads to increased sensitivity to cytotoxic agents as compared to normal tissue.

Specific mutations in DDR genes, whether germline or somatic, could be exploited in planning novel therapeutic interventions. In tumors that have specific defect in DNA repair activity, genomic stability is maintained by upregulation of compensatory DNA repair pathways. This compensatory mechanism may result in resistance to standard chemotherapy, but also in an “Achille’s heel” with specific inhibitors that target the compensatory mechanism or backup survival pathways leading to tumor-specific sensitization.

There is great potential or the targeting of the DDR pathway for cancer therapeutics based on drug development, preclinical efficacy studies and the development of relevant biomarkers to assess the DDR in tumors. An example of basic knowledge translating into clinical therapeutics is the concept of “genetic synthetic lethality” in which the targeting of two related pathways provides increased cell kill of tumors compared to targeting only one of the pathways. Radiotherapy induces various types of DNA damage that is repaired by specific DDR pathways that sense DNA breaks, DNA base damage, DNA cross-links and DNA mismatches. Hence, agents that target a specific DDR pathway may result in reliance of tumor to alternative DDR pathways, which could lead to tumor sensitization. Conversely, pathways that are lost represent vulnerabilities in the DNA repair capacity of the cancer cell that can be exploited by selecting the appropriate chemotherapy and radiation to induce unreparable — and hence more cytotoxic — DNA damage. Indeed, the relative radioresistance of certain tumors but not others, although arrived at empirically, may reflect the relative frequency of a particular DDR defect in that tumor. Therefore, DDR inhibitors have the potential to expand the range of tumor types that can be targeted with radiation and novel DDR agent as long as the therapeutic ratio can be maintained in which late normal toxicity is not also enhanced.

FUTURE POTENTIAL OF THIS AREA OF RESEARCH

Although rare, there have been limited examples utilizing bench to bedside application of targeting DDR pathway molecules. One such example is treatment of BRCA deficient tumors with poly-ADP ribose polymerase (PARP) inhibitors. Tumors with mutations in BRCA1 and BRCA2 have been known to have an underlying defect in homologous recombination required for DNA repair during the S and G2 phase of the cell cycle.3,4 This defect leads to a hypersensitivity to inhibitors of DNA single-strand break and base excision repair and as a result BRCA mutated tumors undergo rapid cell death with single agent PARP inhibitor as secondary back-up survival pathways are inhibited.5 PARP1 is activated by DNA single-strand breaks (SSB) or at stalled replication forks to facilitate DNA repair. The “BRCAness” in these

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tumors is associated with hyper-activation of PARP activity as a compensatory mechanism. Significant interest in PARP inhibitors in BRCA-mutated tumors have led to Phase 1 and 2 clinical trials to explore the overall response rate in breast, ovarian, pancreatic and prostate cancers given a lack of severe toxicity with these agents. A second example of therapeutic manipulation of the DDR is MGMT enzyme expression in patients being treated with temozolomide and radiotherapy for malignant glioma. MGMT is the DNA repair enzyme that removes the cytotoxic O6-guanine adducts produced by alkylator-based chemotherapy, thereby inducing chemoresistance. Greater than 50% promoter methylation had previously been shown to downregulate MGMT and led to biomarker study by SWOG, which showed that there was a significantly divergence in survival based on MGMT expression alone, with survival favoring those patients with low MGMT expression. Based on this result, an EORTC/NCIC trial randomized patients with newly diagnosed glioblastoma following maximal safe resection to standard radiation therapy versus concurrent radiation therapy and temozolomide (TMZ) followed by 6 cycles of post-radiotherapy TMZ. This trial demonstrated an improvement in overall median survival from 12.1 months in the standard radiotherapy cohort to 14.6 months in the TMZ-treated cohort and, of equal importance, an improvement in 2-year survival (27% versus 10%). As a consequence, the TMZ regimen was rapidly adopted as the new standard of care for patients with newly diagnosed glioblastoma.

So what do these 2 trials teach us about advancing the field of DDR in clinical radiation oncology? First and foremost, development of the DDR-associated agents must be based on a strong hypothesis, robust preclinical data in vitro and in vivo, tolerable side effects based on Phase 1 trials and promising efficacy and safety profile in proof-of-concept phase II trials associated with robust biomarkers of the DDR pathways being targeted. Second, a cooperative team of investigators is needed with basic scientists, clinical trialists and the pharmaceutical companies to jointly work together to utilize genomic sequencing data along with novel DDR inhibitors to be successful.

Predictive assay for radiation response: Clinically, it is apparent that there are tumors with increased sensitivity to fractionated radiotherapy which is exemplified by the exquisite radiosensitivity of both normal and tumor tissues in patients with the inherited disorder, ataxia telangiectasia. This sensitivity leads to defects in both DNA repair and cell cycle checkpoint control in patients who have low levels or aberrant function of the ATM gene. It may be that there are other solid tumors that have germline or somatic mutated or differentially-expressed DDR genes leading to increased sensitivity to radiation; but linking these genetic changes to clinical radioresponse requires the use of functional assays to confirm clinical utility of the genetic changes. The successful clinical trials mentioned above were based on ample preclinical evidence showing the underlying defects in DDR pathway. In a similar fashion, increased efforts should made to sub-classify solid tumors in terms of DDR capacity using both genetic and functional assays such that the importance of a given pathway can be assessed for potential DDR targeting in combination with radiotherapy. Robust and validated biomarkers to identify DDR defects that are exploitable by both conventional cytotoxic therapy and agents targeting the DDR are therefore needed to effectively stratify patients.

Incorporation of predictive assays and biomarkers that accurately reflect the DDR into future clinical trials will be vital. For example, activation of the DNA PK and ATM kinases in response to DNA damage can be determined by measuring their autophosphorylation with phospho-specific antibodies, and PARP activity may be measured by immunodetection of the ADP-ribose polymer product. A general marker of DNA damage is the phosphorylation of histone H2AX (referred to as γH2AX) by ATM, ATR and DNA PK. The quantification of discrete nuclear γH2AX foci, formed at sites of DSBs, or increased general nuclear intensity staining for γH2AX, may be measured by immunofluorescence microscopy or flow cytometry to detect DNA damage in the presence of absence of a DDR inhibitor based on ex vivo specimens. The increase and/or persistence of γH2AX can be used to demonstrate the relative radiosensitivity of tumors. Other biomarkers will include tissue staining for overexpressed DDR proteins reflecting compensatory

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up-regulation of DNA repair and activity assays for localized PARP or DNA-PK activity. These assays will also have to be normalized for relative oxygenation status given the effect of tumor hypoxia on the level of initial and residual DNA damage and modification of DNA repair function. Incorporating such biomarker studies early into the design of future clinical trials is critical to understanding tumor DDR biology in situ during fractionated radiotherapy.

In addition, all future clinical trials should incorporate tissue biobanking as part of enrollment criteria in order to facilitate easier access to the tissues in the future. In addition both pre-treatment biopsy as well as post radiation biopsy/resection tissue should also be a part of future clinical trials. Availability of such tissue will be vital to further our knowledge of radiation response.

Early phase clinical trials: One of the frustrating aspects of clinical radiation oncology is the perceived lack of support for early phase clinical trials with novel DDR-associated agents. As compared to medical oncology, there is a general lack of interest in combining these agents with radiation. Although there are numerous preclinical drug developments against almost all the key molecules in DDR pathways, there have been very limited studies that combine radiation with such preclinical targets. What is the reason for the lack of such clinical trials? It is most likely to be multifactorial, but includes the biomarker complexity and the potential toxicity within these trials that decrease sustained enthusiasm by radiation oncologists and big pharma. However, utilizing differential scheduling (e.g. neoadjuvant therapies), genomic and hypoxia profiling and precision radiotherapy with minimal normal tissue volumes can lead to an increased interest in Phase 1 and 2 trials combining radiation and novel DDR targets. This can be aggressively driven by teams acting within and between NCI, RTOG, CTEP, and other collaborative groups. This could over-ride a major limiting factor, which is limited access to promising DDR inhibiting agents to overcome intrinsic radioresistance and improve local control.

Pharmaceuticals: It will be important for ASTRO and other collaborative groups to engage and encourage the pharmaceutical industry to participate in mutual and sustained collaborations. More political support by the ASTRO to promote collaboration between the pharmaceutical and clinical collaborative groups could increase drug access for preclinical and translational science leading to future clinical trials.

Needs within the profession
In the era of limited NIH funding, it is important to use our limited resources with a clear objectives: these include the training of young investigators in the area of DDR targeting to improve radiotherapy efficacy, optimal funding mechanisms for DDR research and setting up real and sustained scientific collaborations between basic scientists, clinical trialists and the pharmaceutical industry. The first objective relates to the training of young PhD and MD investigators and support for an integrative team approach to the understanding of DDR pathways in specific tumor types and a cataloging of important genetic changes that might lead to increased therapeutic response. For this to happen, the DDR pathways must become an integral part of radiation oncology teaching rather than non-clinically related cascades of proteins. Programmatic approaches using specific training conduits (e.g Holman pathways), invitation of trainees to collaborative group meetings, and support to allow scientific visits and experimentation within top DDR research labs even if outside their home institution. Promoting interactions between early stage investigators and outstanding mentors in the DDR field will enhance success in research and funding.

Secondly, we must encourage collaboration between basic scientists and clinical trialists and Big Pharma. Future clinical trials most likely will involve sound benchtop observation and it would be imperative to allow a forum by which benchtop researchers can communicate with clinicians. So how do we accomplish this? One way would be to encourage webinars via internet, workshops within ASTRO, or even encourage inter-institutional journal clubs between clinicians and basic scientists.

There is also potential for ASTRO to broaden its funding mechanisms. Currently there are 2 junior faculty investigator grants and 3-4 resident investigator grants per year. These can be increased with some targeted towards DDR research in which there are outstanding DDR research mentors so as to promote academic careers in the realm of DDR targeting. In the era of limited funding and healthcare cuts, tenure-track academic positions are not as readily available for senior residents. Obtaining a start-up fund for research is even more difficult in this era and sustaining research can be so difficult that attrition occurs in which bright minds are lost to private practice despite initial resource investments. ASTRO should take a leadership role in discussing solutions to this problem with department chairs and strategize how to make more academic positions available to those who have demonstrated clear interest in our field.

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Tumor Metabolism

Current status
Sixty years ago Warburg observed that the rate of glycolysis is abnormally high in cancer cells even though the amount of glucose used for oxidative phosphorylation is much less than in normal cells. Recent work has offered an explanation for this apparent anomaly in the large biosynthetic requirements of tumor cells for proliferation. The metabolism of cancer cells also demonstrates other characteristics that distinguish it from metabolism of normal cells including a reliance on “glutamine addiction” necessitated by the need for amino acid precursors and enhanced fatty acid synthesis necessary for membrane biogenesis. The oncogene-driven mechanisms that drive these metabolic changes remain poorly defined. However, the recognition that aerobic glycolysis is not simply an adaption to hypoxic environments has reignited interest in the relationship between oncogenes and metabolism. Thus the growth factor receptor driven PI3k-Akt-mTOR pathway has been shown to increase expression of HIF-1α. This transcription factor stimulates the expression of glucose transporters and almost all the enzymes involved in the glycolytic pathway. In addition HIF-1α by stimulating expression of pyruvate dehydrogenase kinase isozymes inactivates pyruvate dehydrogenase inhibiting pyruvate entry into the tricarboxylic acid cycle (TCA) and as a consequence inhibiting oxidative phosphorylation. The transcription factor c-Myc whose expression is altered in 70% of human tumors regulates the expression of several key metabolic enzymes including proteins involved in glutamine metabolism, for example membrane transporters and a mitochondrial glutaminase. This latter is essential in the conversion of glutamine to glutamate. The most studied tumor suppressor gene, TP53 is also intimately involved in cell metabolism. For example wild type TP53 suppresses GLUT1 and GLUT4 gene expression whereas certain TP53 mutants are ineffective repressors of glucose transporter expression.

Besides these extensively studied pathways, mutations in genes encoding mitochondrial proteins that facilitate cancer cell metabolism have been identified in diverse cancer cells. Succinate dehydrogenase (SDH) forms complex II of the electron transport chain and couples the oxidation of succinate to fumarate in the TCA cycle to electron transport. Mutations in genes encoding subunits of this enzyme as well as its reduced expression have been found in several different cell types. Reduced SDH activity and as a consequence elevated succinate levels inhibits HIF-1α prolyl hydroxylase thereby elevating HIF-1α levels. Mutations in the gene encoding another TCA protein, fumarate hydratase (FH), have also been identified in certain cancers and result in reduced FH activity. As with the SDH deficiency this results in increased HIF-1α levels. Mutations in the isocitrate dehydrogenase, IDH1, are also thought to result in increased HIF-1α levels although there is some evidence for regulating NADPH synthesis as well.

Current resources
Some measure of current resources in this area can be obtained by querying NIH Reporter using the key words radiotherapy and glycolysis, radiotherapy and energy metabolism and radiotherapy and mitochondria. The numbers of extramural grants (and an estimate of investigators directly involved) of all types (R01, R21, R03, F and K series) are respectively 7, 10 and 54 for the three key word searches. The search results for radiotherapy and mitochondria is misleading in the context of metabolism since they include numerous grants where apoptosis is measured with no direct interest in metabolism. The very substantial differences between normal and tumor metabolism argue that more emphasis should be placed on research exploring these differences to enhance the anti-tumor effectiveness of radiotherapy.

Future potential
Because tumor radioresistance is in part due to hypoxic fractions within tumors, one would predict that therapies targeting hypoxia and the requirement for glycolysis in tumor metabolism would at least be additive in anti-tumor effectiveness with radiation. Because of the established relationship between the Warburg Hypothesis and cancer, a number of strategies have been developed to target key enzymes in glycolysis. Most of these studies, however, have not been in combination with radiotherapy. Glycolytic inhibitors in various stages of clinical testing include 2-deoxyglucose, 3-bromopyruvic acid, lonidamine, and TLN-232.1-3 In general their relative anti-tumor effectiveness as single agents has been disappointing. More recent studies have explored combined therapies. The rationale for combining a glycolytic inhibitor such as 2-deoxyglucose with radiotherapy (Continued on next page)
derives from the energy requirements for DNA repair that in tumor cells are dependent on aerobic glycolysis. A recent review of several Phase I/III trials in India suggests a benefit from including 2-deoxyglucose with radiotherapy in the treatment of glioblastomas. A search of clinicaltrials.gov for 2-deoxyglucose or any of these other anti-glycolysis agents in active trials with radiotherapy was negative.

Temsirolimus and derivatives, inhibitors of the PI3k/Akt/mTOR pathway, are being evaluated in several trials with and without radiation and have obtained FDA approval for treatment of renal cell carcinoma. Phase I trials testing combined chemoradiation with mTOR inhibitors have established reasonably tolerated Phase II dosage. FDG positron emission tomography indicated that in a subset of patients changes in tumor metabolism were observed within days of initiating treatment.

Another approach has been to use dichloroacetate (DCA) to inhibit pyruvate dehydrogenase kinase thereby activating pyruvate dehydrogenase and shifting cancer cell metabolism from aerobic glycolysis to oxidative phosphorylation and glucose oxidation. In vitro work, animal studies and clinical investigations have suggested the antitumor efficacy of DCA. A Phase I study with glioblastoma patients prospectively obtained tumor tissue before and after DCA treatment. Analysis of the tissue demonstrated that DCA treatment depolarized mitochondria, enhanced mitochondrial reactive oxygen generation and increased tumor cell apoptosis. In addition, DCA reduced HIF-1α expression, promoted p53 activity and was anti-angiogenic. These biochemical and cellular changes are consistent with its mode of action as defined in vitro. At the orally given doses, it was also well tolerated with the dose limiting toxicity, reversible peripheral neuropathy. Presently there is a randomized masked placebo-controlled single-center study to evaluate the effects of DCA versus placebo given in combination with cisplatin and radiation treatment in patients with Stage III-IV Head and Neck Squamous Cell Carcinoma (NCT01386632). A previous in vitro study demonstrated that DCA sensitized both wild type and Bcl-2 over-expressing prostate cancer cells to radiation. A potential drawback for combining with radiotherapy is that by increasing glucose oxidation and oxygen consumption, DCA might actually make tumors more hypoxic and thus more radioresistant, a result that would not be discovered in vitro. Nonetheless, the preclinical work demonstrating anti-tumor efficacy with various tumor types, its 100% bioavailability, relatively low toxicity, the low price (it is a generic drug) and that DCA has been used in humans for more than 30 years, strongly argues for rapid clinical translation including in combination with radiotherapy.

Recent investigations indicate an intriguing relationship between a key regulatory enzyme of glycolysis, AMP kinase (AMPK), and the double strand break repair kinase, ataxia-telangiectasia mutated (ATM). AMPK is an effector of the tumor suppressor gene LKB1 that when mutated in Peutz-Jeghers syndrome is associated with susceptibility to lung, pancreatic and breast cancer. AMPK is activated by AMP binding at low energy conditions such as induced by hypoxia or nutrient deprivation or by phosphorylation by kinases such as LKB1. Active AMPK enhances cellular energy levels by stimulating glucose uptake and glycolysis but also by phosphorylating p53 at Ser15 initiating a metabolic checkpoint and inhibiting anabolic pathways such as fatty acid and cholesterol biosynthesis necessary for cell proliferation. Ionizing radiation also stimulates AMPK activity in human cancer cells by a mechanism independent of LKB1 but abrogated by KU-55933, an inhibitor of ATM. In vitro studies further show that the drug metformin used in the treatment of diabetes and an activator of AMPK is a radiosensitizer whereas an inhibitor of AMPK, Compound C, is a radioprotector. Additional potential interest are the findings that activating AMPK arrests endothelial cell proliferation through phosphorylation of TP53 and cell cycle arrest with enhanced p21/p27 expression. AMPK activation also blocked the migration of endothelial cells in a scratch assay but stimulated tube formation in matrigel. This suggests that activating AMPK may be an approach to normalizing vasculature in diseases such as cancer that are characterized by aberrant endothelial growth. Thus, these results present

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Another approach has been to use dichloroacetate (DCA) to inhibit pyruvate dehydrogenase kinase thereby activating pyruvate dehydrogenase and shifting cancer cell metabolism from aerobic glycolysis to oxidative phosphorylation and glucose oxidation. In vitro work, animal studies and clinical investigations have suggested the antitumor efficacy of DCA.
a therapeutic maneuver to achieve Jain’s concept of normalizing tumor vasculature and reoxygenating tumors for radiosensitization. Animal studies preferably with spontaneous tumors to better mimic the microenvironment of tumors are needed to test these mechanisms for radiosensitization.

Studies on individualizing anti-metabolic treatment strategies in HNSCC have emphasized the importance of the genetic component in the effectiveness of any molecular targeting therapy. Glycolytic flux, mitochondrial respiration and relative radiosensitivities were evaluated in several HNSCC cell lines of known TP53 mutant or wild type status. TP53 mutation was associated with the metabolic shift to glycolysis and increased radiosensitization to glycolytic inhibitors such as 2-deoxyglucose. The observed glycolytic dependence was due to decreased mitochondrial complex II and IV activities. Wild type TP53 cells were relatively insensitive to 2-deoxyglucose. Both inhibitors of mitochondrial respiration (metformin) and glycolysis were required to sensitize wild type TP53 HNSCC cells to radiation. TP53 mutants were further stratified in terms of whether the mutation was disruptive, defined as resulting in a truncated protein or in the DNA binding domain causing a change in polarity within the protein, or non-disruptive to overall protein structure. Disruptive TP53 mutations and not mutant TP53 status alone predicted locoregional recurrence in 74 patients retrospectively followed. Furthermore, cell lines with disruptive TP53 mutations were significantly more radioresistant demonstrating reduced radiation-induced senescence. In studies using HNSCC xenografts, it was determined that metformin sensitized tumors carrying disruptive TP53 mutations at least in part by enhancing radiation-induced senescence. Furthermore a retrospective analysis of those 74 patients showed that locoregional recurrence was decreased in patients taking metformin. These studies demonstrate the potential for using anti-metabolic treatments to enhance the efficacy of radiotherapy but also re-enforce the need to define the genetic character of the target to maximize the effectiveness of the anti-metabolic therapy.

**Needs to progress this field**

Further advances in targeting energy metabolism of tumors in combination with radiotherapy can be achieved in several different areas.

a) Glycolytic inhibitors by themselves are not very effective. For example, inhibition of glycolysis by 2-deoxyglucose is readily reversible by increasing glucose. Appropriate combinations of metabolism-targeted drugs such as described above with 2-deoxyglucose and metformin need to be further developed. Another combination of drugs that is worthy of consideration is statins with metformin. Both are anti-inflammatory but have different targets. Statins may also reduce the normal tissue toxicity of radiation. Other targets especially those that have potential in being tumor specific need to be defined. For example, prostate cancer cells do not accumulate Zn2+ as do normal prostate cells and this frees up citrate for the TCA cycle. The underlying mechanisms appear to be reduced expression of Zn2+ import transporters and increased expression of Zn2+ exporters. This suggests a strategy to interfere with prostate tumor energy metabolism by increasing Zn2+ uptake by targeting either the import or export processes.

b) Effects of anti-metabolics on supporting stromal cells need study. Although these cells are not necessarily “normal”, for example, tumor endothelial cells express ERBB1 in contrast to normal endothelial cells that express ERBB4, they retain their TP53 wild type status. The energy metabolism of these cells is rarely investigated. As discussed above, endothelial cell proliferation is blocked and differentiation enhanced with tube formation with metformin suggesting one mechanism to normalize tumor vasculature. The effects of these anti-metabolites on the inflammatory cell compartment of these tumors needs to be evaluated since these cells generate much of the reactive oxygen and reactive nitrogen and growth factors that promote chromosomal instability and tumor cell proliferation.

c) All future clinical trials investigating the interactions of anti-metabolic treatment strategies with radiation should have a genetic component. As the HNSCC studies demonstrate knowledge of TP53 mutant status may very well predict what strategy is used. Information on mutational status of different genes known to impact cell energy metabolism (e.g. c-myc, sdh, idh1) or on normal polymorphisms in genes that impact gene expression levels or activities should be developed. Ideally, a requirement for these studies would be to obtain the buffy coat from all consenting patients. A genetic stockpile with the necessary patient information would potentially provide a goldmine for evaluating different treatment strategies.

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Current status

There are four major areas in which molecular imaging and nanotechnology are being applied in radiation oncology research. The first is the identification of molecular phenomena of interest in radiation oncology, and generation of probes, nanoparticles, and imaging methods targeting these phenomena. Many physiologic and molecular aspects of cancer may be of use in molecular imaging, including angiogenesis, perfusion, hypoxia, and metabolism, as well as expression of tumor-specific genes and proteins. Imaging approaches targeting these processes are in many cases well established in the molecular imaging field. For example, a variety of nuclear medicine techniques for imaging tumoral hypoxia have been developed over the past 20 years and are now in clinical use in a number of research centers.\(^1\) Magnetic resonance imaging (MRI) and x-ray computed tomography (CT) methods for imaging tumor perfusion are now commonly available on commercial imaging systems. Protein-specific techniques have generally focused on cell-surface proteins and receptors such as epidermal growth factor receptor (EGFR), carbonic anhydrase IX (CAIX), and matrix metalloproteinases (MMPs) because of their access to exogenous imaging agents.

The second major area is the design of therapeutic and theranostic agents based on nanomaterials. While a variety of nanoparticles have been developed as vehicles for imaging probes, these materials have also been applied toward development of therapeutic agents. This includes both use of nanomaterials as scaffolds to which therapeutics including cytotoxic drugs, prodrugs, radioisotopes, and protein inhibitors are coupled, as well as the so-called “theranostic” agents that are capable of both providing imaging information on tumor status as well as delivering a therapeutic payload. A vast array of therapeutic and theranostic nanoparticles have been reported in the literature, and have shown to be effective in preclinical studies. Tumor-specific uptake of nanoparticles can be facilitated through both passive targeting by the enhanced permeability and retention (EPR) effect as well as by incorporation of molecular targeting strategies.

The third area is the development of methods for including molecular imaging in the staging and radiation treatment planning processes. Biologically-conformal radiation therapy (BCRT) was first proposed by Clif Ling in 2000 in order to apply the wealth of biological information now available through molecular imaging and other procedures towards optimizing the delivery of radiation treatments.\(^2\) A number of groups have devised methods for segmenting functional and molecular imaging datasets for use in delineating target volumes, including positron emission tomography (PET)\(^3\)\(^-\)\(^4\) and magnetic resonance spectroscopy (MRS).\(^5\) An application of molecular imaging that is less dependent on a precise spatial mapping of the target is use of these methods to stage cancers and select an optimal treatment course. For example, the application of stereotactic body radiotherapy (SBRT) may be limited by tumoral hypoxia, and efforts are underway to assess the ability of hypoxia imaging methods to predict response to SBRT and accordingly to select patients in which this treatment will be effective.

A fourth area is early detection of tumor response to radiation. Current measurement of the outcome of cancer therapies including radiation treatment focuses on changes in tumor volume measured by anatomic modalities such as CT and MRI.\(^6\) However many of the biological processes cited in area 1 as targets for molecular imaging are known to be altered at timepoints following radiation that precede gross changes in tumor size. In addition to early detection of tumor response, molecular imaging methods have been shown to identify immediate changes following radiation allowing their use to verify dose delivery, particularly for particle therapy.\(^7\) Similarly, a number of molecular imaging methods are sensitive to radiation-induced toxicities in normal tissue, such as radiation-induced inflammation and bone marrow suppression.

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Current resources
Molecular imaging is a technologically-intensive endeavor involving interactions between physicists, engineers, chemists, biologists, and clinicians, and this has limited its deployment. While MRI as well as PET using standard, commercially available radiotracers are now practiced routinely in a number centers, investigation of novel imaging agents and imaging procedures are generally limited to a handful of sites with established molecular imaging programs. In addition, as molecular imaging programs generally arise from radiology programs, funding and personnel for applications of these methods in radiation oncology have been sparse.

Future potential
While research continues in the four areas identified in the preceding section, the incorporation of molecular imaging and nanotechnology into clinical radiation oncology is at a crossroads. The key issues faced by each of these endeavors and their potential for long term success in transforming the field of radiation oncology are discussed below.

1. Identification of molecular phenomena of interest in radiation oncology, and generation of probes, nanoparticles, and imaging methods targeting these phenomena
A wealth of molecular probes with applications in radiation oncology have been generated, and methods for synthesis of novel probes based on existing targeting agents and imaging groups are now standardized. The focus of future research must be on translation of these agents into clinical practice, and demonstration of their utility in prospective clinical trials. This does not preclude development of new agents, however the translational “loop” must be closed so that the current crop of agents are properly vetted through clinical studies, and shortcomings of these agents are subsequently addressed through new probe design and preclinical studies that will similarly lead to clinical evaluation.

A primary concern in the translation of molecular imaging probes to clinical use is the relatively poor signal-to-noise ratios (SNRs) afforded by these imaging methods. For example, current PET radiotracers for hypoxia in general give a maximum SNR of approximately 4, resulting in imaging “thresholds” for hypoxia on the order of 1.2 – 1.5. Given the number of sources of error in PET as well as in other molecular imaging modalities, including variability in acquisition and data analysis, this poor SNR is tremendously problematic for successful clinical application of these technologies. This shortcoming presents both an opportunity for development of new molecular imaging probes with improved SNR, either through incorporation of signal amplification strategies or through selection of more robust targets, as well as development of standardized imaging protocols to manage the sources of error associated with these methods and allow productive multi-institutional imaging-based trials, as discussed below.

2. Design of therapeutic and theranostic agents based on nanomaterials
While novel developments in materials science have produced a variety of nanometer-sized structures with exciting potential for biological applications, their incorporation into clinical medicine has been slow. One of the chief limitations has been obtaining regulatory approval for clinical studies of nanomaterials, often containing inorganic and sometimes toxic components, that may have a long residence time in the body. This continues to be a bottleneck for translational nanoparticle research, and can only be addressed through 1) comprehensive demonstration of the safety profile of nanoparticle agents, and/or 2) development of organic nanomaterials with reduced safety concerns. A second limitation of current nanotechnology work is the reliance on these materials as scaffolds for targeting using current methods such as antibodies or receptor ligands, imaging using conventional magnetic, radioactive, or fluorescent groups, or therapy using existing cytotoxic drugs. While this is attractive from a chemical synthesis standpoint, it raises the question of how these agents will outperform non-nanotechnology imaging and therapeutic strategies employing the same components. In order to exploit the full potential of nanomaterials, the groundbreaking physical properties of these devices must be the focus. Because nanomaterials do not inherently home to tumor more so than conventional drugs or imaging probes when delivered systemically, imparting improved specificity to these agents may be achieved by one of two mechanisms. Continued improvements in antibody or ligand targeting may be pursued, but more intriguing is giving the nanodevices themselves the capability to be biologically specific. Development of nanomaterials with properties of use in radiation oncology, such as nanophosphors that absorb high energy radiation and convert this energy into another form such as cytotoxic low energy electrons or low energy photons for imaging, is of prime interest.

3. Development of methods for including molecular imaging in the staging and radiation treatment planning processes
The clinical challenge of extracting the biologic information required for BCRT from a limited number of imaging studies has proven challenging. Molecular imaging in radiological applications commonly amounts to a
"yes/no" question of whether a certain signal is observed in the target, and whether by association a certain biological process is occurring there. In radiation treatment planning the question is spatial: for example delineating the boundary between normal and neoplastic tissue, or identifying a volume for delivery of a boost dose of radiation. Rigorous validation of the volumes produced by proposed methods has been difficult, and this has slowed their deployment in standardized clinical trials. Careful studies of the reproducibility and robustness of target volumes generated by molecular imaging methods are sorely needed.

This problem encourages two types of solutions, one a “bottom up” approach focused on improving molecular imaging methods so that specific biologic parameters may be measured as discussed in item 1 above, and the other a corresponding “top down” strategy in which clinical radiotherapy based on established molecular imaging methods such as fluorodeoxyglucose (FDG) PET is developed based on empirical evidence but without a rigorous model of the procedure at the cellular or molecular level. As commonly is the case in such situations, the effective path forward lies in combining the top down and bottom up paradigms to meet in the middle. With the recent introduction of preclinical image-guided conformal radiotherapy systems, there are now tremendous opportunities to explore strategies for molecular imaging-guided radiation therapy in the laboratory prior to clinical translation.

Perhaps the biggest benefit to be gained from incorporating functional imaging into the treatment planning process is improved definition of the tumor volume to incorporate neoplastic regions not easily identified on anatomic CT or MR imaging. Identification of tumors that are particularly radiosensitive or radioresistant by molecular properties is also an imminent and useful clinical possibility. This concept may be extrapolated towards Ling’s vision of BCRT by building biologic models of tumor genesis and progression combining anatomic imaging, molecular imaging, and cancer biology for use in defining a radiation target. Such an approach may take into account the uncertainty inherent in current low signal-to-noise and spatial resolution imaging methods. Modulating or boosting the dose to the GTV or subsets of it based on biologic information is an exciting possibility, but given the physical realities of radiation dose delivery this development may only marginally improve overall clinical outcome. Part of this pessimism can be attributed to the disconnect between microscopic, cellular radiobiological models and macroscopic tissue imaging methods.

4. Early detection of tumor response to radiation

While imaging of functional parameters such as vascu-

larity, metabolism, and cellular proliferation are now widely possible with dynamic contrast magnetic resonance imaging (DCE MRI) and perfusion CT, diffusion-weighted MRI (DW-MRI), FDG PET, and fluorothymidine (FLT) PET, the application of these methods towards measuring radiation treatment outcome has been limited, particularly in the clinical setting. This is in part due to the lack of standardized metrics for assessing response, a problem related to those discussed in item 1 above. Moreover, image registration is essential for identification of longitudinal changes in molecular imaging signals after radiotherapy, and the lack of standardization of these methods is similarly problematic.

Future developments in this area of molecular imaging research will share a similar theme to that identified in item 3 above, the merging of “top down” and “bottom up” approaches. “Bottom up” research will focus on the development of new imaging probes sensitive to specific aspects of tissue and cellular radiation response. Molecular imaging agents reporting on intracellular targets have been challenging to achieve, however renewed focus in this area is required to develop noninvasive imaging strategies for well-established histologic radiation damage markers such as γH2AX. Systematic and comprehensive evaluation of existing molecular imaging methods such as those cited above will comprise the “top down” approach. These efforts are particularly timely with the emergence of adaptive radiotherapy, and may synergize with this treatment approach that has been frequently discussed but has not as yet been implemented or evaluated clinically in a standardized fashion.

Needs within the profession

At present, there is tremendous variability both within and across sites in terms of the methods for acquiring and analyzing molecular imaging data. This is a problem that is common to molecular imaging in both radiology and radiation oncology. For example, accurate application of PET imaging requires measurement and decay correction of the injected dose and imaging data, standardization of the time between radiotracer injection and imaging, and routine quality assurance measures to calibrate image intensities measured by the system. The variability associated with nanoparticle synthesis, even within a single center, have been well reported and present a substantial barrier to both characterization of these agents as well as regulatory approval for clinical studies. Furthermore, methods for image quantitation are poorly standardized. Region-of-interest (ROI) based quantitation is

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prevalent, but protocols for reproducibly defining ROIs are not well established. Similarly, many quantitative metrics can be extracted from ROIs, and reports in the literature are not consistent as to which metric is reported. Estimates of the effects of these sources of variability have been reported to be as high as ±50% in PET, which severely limits the useful conclusions that can be extracted from multicenter trials. At present, a number of efforts are underway to establish standardized protocols for quantitative imaging, including the National Cancer Institute-sponsored Quantitative Imaging Network (QIN) and the American College of Radiology Imaging Network (ACRIN). Radiation oncology efforts have some representation in these programs, which should be expanded to ensure consideration of quantitative issues specific to this discipline, such as volume delineation.

As discussed above, a great many molecular imaging strategies with applications to radiation oncology have been reported in the literature. However, while retrospective studies have suggested that many of these techniques have promise in clinical radiation oncology, there has been an overall lack of rigorous, controlled, prospective clinical trials to evaluate their performance in this context. This shift in approach for imaging research in radiation oncology is needed both within and across institutions in order to fully implement molecular imaging in this field.

A significant barrier to progress in applying molecular imaging to clinical radiation oncology is the necessity of cross-departmental collaboration. While modern radiation oncology is inherently image-based, molecular imaging methods remain primarily rooted in radiology departments and few radiation oncology centers have the capacity to acquire their own molecular imaging data or the expertise required to analyze it. It is not feasible or even advantageous for a radiation oncology department to construct its own molecular imaging center. Collaborations with radiology and molecular imaging programs are therefore crucial, but the practical barriers can be substantial. Funding for imaging studies within radiation oncology is a critical problem, including both support for imaging research in this field as well as general mechanisms for supporting imaging examinations outside of radiology departments. Issues of this type to be overcome include the cost of imaging agent synthesis and radiochemistry and distribution of scan revenues. This problem encourages activism within the radiation oncology community to campaign for increased funding for molecular imaging studies. In addition, radiation oncology training programs must incorporate imaging so that the next generation of leaders in this field can more effectively conduct this increasingly multi-disciplinary research.

**Infrastructure needs**

- Funding sources to support investigation of molecular imaging methods in clinical radiation oncology. Ideally these funding mechanisms would be tied to adoption of the standardized imaging protocols discussed above.
- Improved and expanded distribution networks for radiotracers, nanoparticles, and other molecular imaging agents to allow simple and efficient access to novel imaging methods.
- Incorporation of support for molecular imaging modalities into clinical radiation oncology software, including treatment planning systems.

**CHAPTER REFERENCES**


Current status

Normal tissue stem cells possess the unique ability to generate new stem cells through a process termed self-renewal and to differentiate into the specialized cells of an organ. Cells cultured in vitro, which demonstrate self-renewal and differentiation, are putative stem cells. However, to formally demonstrate that a cell is a stem cell, in vivo studies are required.

There are two in vivo approaches to establish stem cell identity: lineage tracing and single-cell transplantation. For lineage-tracing experiments, the Cre-loxP system is utilized to express a reporter protein, such as β-galactosidase or green fluorescent protein, specifically in the putative stem cell compartment. Because activation of the reporter protein occurs by recombination of genomic DNA, all of the putative stem cells and their progeny will express the reporter protein. Therefore, by following the expression of the reporter protein in vivo, lineage-tracing experiments define whether a specific cell population renews the stem cell compartment over time and whether these cells give rise to more differentiated cells within the organ. For example, Barker and colleagues utilized lineage tracing to identify the Lgr5+ crypt base columnar cells as stem cells in the small intestine. To establish stem cell identity by transplantation, a single putative stem cell, which is labeled by a reporter protein, can be transplanted into a recipient animal in which the organ has been removed or ablated. Then, the capacity for the putative stem cell to regenerate the entire organ can be assayed. For example, Shakleton and co-workers used cell surface markers to isolate Lin-CD29hiCD24+ cells from the mouse mammary gland to show that a single mammary stem cell can reconstitute a complete mammary gland in vivo.2

The identification of stem cells in normal tissues has important implications for understanding, preventing and treating radiation toxicity. In many tissues, acute and late effects of radiation are a consequence of the depletion of resident tissue stem cells. Therefore, by defining tissue-specific stem cells, it may be possible to design drugs or other therapies that prevent their depletion by radiation or promote their regeneration. These approaches may protect against or mitigate radiation injury.

The identification of stem cells in normal tissues has also led to advances in our understanding of cancer development. To investigate the cell-of-origin of cancer, investigators have used genetically engineered mice to turn on gene mutations specifically in stem cells or more differentiated progeny. For example, mutation of APC in Lgr5+ stem cells leads to intestinal cancer, while the same mutation in transit amplifying cells does not. However, with the right constellation of gene mutations, differentiated cells can gain stem cell-like properties and initiate tumorigenesis. For example, mutation of APC and K-ras in the differentiated villus epithelium causes dedifferentiation into intestinal tumor cells. Therefore, tumor initiation may occur in a stem cell or a more differentiated cell. In both cases the tumor cells co-opt stem cell signaling pathways of self-renewal for tumor maintenance. Therefore, targeting these signaling pathways of self-renewal is an attractive therapeutic approach.

A better understanding of the hierarchy of normal tissues from stem cells to differentiated cells has led to new models for understanding tumor cell heterogeneity. While radiation biologists have long recognized that different cells within a tumor maintain different capacities for clonogenic survival after transplantation or irradiation, this concept has been adapted into the framework of stem cell biology in the cancer stem cell model. In this model, cancer heterogeneity is due to the presence of a small subset of cancer cells, which are endowed with the stem cell properties of self-renewal and the capacity to differentiate into non-clonogenic cancer cells. (Continued on next page)
The identification of cancer stem cells in human cancers has typically been performed by isolating populations of cancer cells by the presence of specific cell surface markers by flow cytometry and then transplanting these cells into recipient immune-deficient mice. Using this approach, investigators have identified cancer stem cells in a variety of human cancers. Importantly, several studies suggest that these cancer stem cells may be resistant to radiation therapy. For example, Bao and colleagues isolated CD133-positive cancer stem cells from gliomas and observed that after irradiation these cells preferentially activate the DNA damage response and repair DNA damage compared to CD133-negative cancer cells. Therefore, these investigators proposed that the CD133-positive cancer stem cells confer radiation resistance to gliomas. Diehn and co-workers reported that compared to other cancer cells, cancer stem cells in human breast cancer contain lower levels of reactive oxygen species. Therefore, these authors proposed that the low levels of reactive oxygen species in cancer stem cells may contribute to radiation resistance in breast cancer.

**Future potential**

Stem cell biology underpins normal development, tissue homeostasis, and regenerative medicine. To advance these disciplines, we anticipate important advances in stem cell biology in the future. This work has the potential to be translated into radiation oncology to impact the clinic by increasing the therapeutic window. First, the application of knowledge of stem cell biology can be used to ameliorate normal tissue toxicity from radiation therapy. For example, the radiation-induced gastrointestinal (GI) syndrome is regulated by the p53 pathway in GI epithelial cells. With the identification of Lgr5+ stem cells in the small intestine novel therapeutic approaches that improve the survival of these stem cells following irradiation or promote their regeneration after irradiation may decrease radiation toxicity to the small intestine. However, drugs that promote the proliferation or survival of normal tissue stem cells have the potential to promote tumor cell repopulation during or after radiation therapy. As discussed above, cancer cells often hijack mechanisms of self-renewal that are used by normal tissue stem cells. Therefore, as therapies are developed to promote the recovery of normal tissue stem cells following radiation therapy, it will be important to characterize how these therapies impact tumor cure following radiation therapy. One approach to promoting the regeneration of stem cells after radiation therapy that may not impact cancer cells is stem cell transplantation. For example, Nanduri and colleagues have isolated stem cells from the mouse salivary gland. Intriglandular injection of these cells into the salivary glands of irradiated mice promoted recovery of salivary gland function. Therefore, the application of stem cell biology to clinical radiation oncology via pharmacological or stem cell transplant approaches has great potential to decrease acute or late toxicity from radiation therapy.

Advances in stem cell biology also have the potential to impact clinical radiation oncology by providing the opportunity to specifically target radioresistant cancer stem cells to improve cure rates with radiation therapy. Recent studies with genetically engineered mice have utilized lineage tracing of specific populations of glioma cells to not only identify cancer stem cells in vivo, but to also identify a population of cells that propogates glioblastoma after temozolomide chemotherapy. Similar pre-clinical studies with radiation therapy in a variety of cancers should be prioritized. These experiments may identify specific cell populations, which can be targeted by drugs given concurrently with radiation therapy to improve rates of local control.

**Needs within the profession**

The opportunity to leverage the advances of stem cell biology into improved outcomes for patients treated with radiation therapy is large. To maximize this opportunity, it will be critical for radiation oncologists to be well versed in advances of normal tissue stem cell biology and cancer stem cells. Radiation oncologists with stem cell biology training will be well-positioned to perform translational studies to try to mitigate radiation toxicity and/or improve local control. Alternatively, radiation oncologists may partner with stem cell biologists to identify areas within stem cell biology that are ripe for translation. Because stem cell biology is such a fundamental area to so many other important scientific and clinical disciplines, further advances in stem cell biology outside of radiation oncology will proceed regardless of whether this new knowledge is translated into gains in our field. One challenge to radiation oncology is whether we will be able to fully seize the opportunity of stem cell biology by performing equally sophisticated and elegant studies to study cancer stem cells with radiation therapy that others have performed with radiation therapy. A second challenge for the next decade will be to translate the discoveries of cancer stem cells and normal tissue stem cells into clinical trials to widen the therapeutic window of radiation therapy.

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CHAPTER REFERENCES


Current status of research

Radiation has historically been viewed as immunosuppressive, despite evidence that it has both pro and anti-inflammatory effects. The immunosuppressive application is in regards to total body irradiation for transplants. Despite the well-known contribution of the immune system to the response to radiotherapy, interest in selectively exploiting the effects of radiation on the immune system have only emerged in the past 10 years. Advances in cancer immunotherapy and publication of well documented immune mediated abscopal effects (distant responses to local therapy) have helped raise awareness of the potential in this field. While the optimal field and dose/fractionation of radiotherapy remains undefined, when combined with immunotherapy, high dose focused radiation techniques like stereotactic body radiation (SBRT) are currently under investigation in trials that combine novel immune modulating drugs and cancer vaccines. This clinical interest is paralleled by a rejuvenated interest in the biology of the immune response to radiation.

Our knowledge of the immunosuppressive and immunogenic effects of radiation remains incomplete, but several determinants are emerging. These factors include the dose, fractionation, field size, and inherent immune responsiveness of the patient. Large fields or whole body radiation treatments are primarily immunosuppressive through effects on lymphocytes, which undergo programmed cell death at comparatively low doses of radiation. Suppression of T cells is critical for effective transplant engraftment and is the main objective of total body irradiation. T cells have subtypes including effector T cells and regulatory T cells that are generally pro-inflammatory and immunosuppressive, respectively. While most radiation related immunosuppression is a result of destroying effector T cells, there is evidence that regulatory T cells are more resistant to radiation, thus potentially altering the balance of T cell subtypes towards immunosuppression. Similarly, low dose radiation to a local area can dampen inflammatory responses. This strategy has been exploited sporadically for treatment of local symptoms in benign chronic inflammatory and fibrotic diseases such as rheumatoid arthritis and Dupuytrens contracture.

Even in the immunosuppressive context of total body irradiation, pro-inflammatory responses such as radiation pneumonitis may occur. These involve the innate immune system, with epithelial cell death and the alveolar macrophage response playing a central role in this significant toxicity. Most current pneumonitis research has focused on defining the critical cytokines for radiation pneumonitis. Preclinical mouse experiments of radiation induced lung damage have shown the importance of IL-1 and TNF-α in the inflammatory phases. Radiation activation of TNF-α induction appears to be regulated at the RNA stability level by tristetraprolin. TGF-β has strong evidence for a role in the post-inflammatory fibrotic response. The precise molecular roles of these cytokines including their target cells remains poorly defined. While promising, use of peripheral blood cytokines as biomarkers for selection of patients at risk for radiation pneumonitis has been disappointing.

While much radiobiology research has focused on understanding and mitigating normal tissue inflammatory toxicities of radiation, a new research field has emerged aiming to harness radiation to stimulate cancer immunity. Research studies have coalesced on several specific areas: 1) defining the cell subtypes responsible for sensing and triggering a radiation related immune response, 2) characterizing the cell surface receptors and cytokines important for these interactions, 3) understanding the mechanisms of immune cell recruitment to the radiated site, 4) characterizing the radiation induced antigens and their processing and presentation by professional antigen presenting cells.

Radiation helps elicit anti tumor responses that are dependent on T lymphocytes and professional antigen presenting cells called dendritic cells. The use of syngeneic mouse models and antibody depletion of immune cell sub-types provides strong evidence of radiation-induced immune responses that are responsible for abscopal effects. The relative contributions of other immune cells and various T cell subtypes is an area of current investigation.

The immune system is constantly on alert for “danger” signals to determine when an immune response is appropriate. Cell damage and death caused by radiation elicits this danger signal that can activate the innate immune system. The type and timing of tumor cell death is critical to eliciting an effective cancer specific immune response. The historical view that apoptotic cell death is immunologically silent (Continued on next page)
while necrotic death is immunogenic has been challenged by reports suggesting apoptosis can be immune stimulating. During cell death, translocation of the endoplasmic reticulum molecule Calreticulin on the plasma membrane has been demonstrated to be a requirement for immunogenic cell death (ICD).\(^8\)\(^,\)\(^12\) Moreover, radiation leads to expression of damage associated molecular patterns (DAMPs) such as HMGB1, monosodium urate, and ATP, both required steps for ICD\(^8\). Some evidence exists that higher dose fractions tend to be more immunogenic, however it is unclear whether multi-fractionation regimens are required. The choice of dose and fractionation may depend on the type of immunomodulator chosen to be combined with radiotherapy.\(^9\)\(^,\)\(^13\)

An adaptive immune response depends critically on T cell recognition of tumor cell antigen presented by professional APCs. Radiation can alter tumor antigen processing and expression on the surface of tumor and dendritic cells (DC). For effective T cell responses against these antigens they must be presented in the context of MHC and a second cell surface signal must be provided. Irradiated cells express increased MHC and costimulatory molecules in response to radiation.\(^14\) Irradiation also stimulates neoantigen expression by altering the peptide processing of antigens, and increasing oncofetal gene expression.\(^15\) The combination of these factors lead to improved antigen presentation, costimulation, and successful T cell activation after local radiation.

Established tumor microenvironments generally lack adequate signals for immune cell homing and trafficking: evidence exists that radiation improves immune responses by recruiting immune cells to the tumor. While the antigen uptake and cross presentation phase is essential to enable activation of T cells in the lymph nodes, cytotoxic T cells need to successfully migrate to the tumor to be effective. Radiation causes the release of cytokines, such as RANTES, Gro-1, MCP-1, and CXCL\(^16\) leading to recruitment of T cells. Irradiation also increases expression of selectins on endothelial cells leading to adherence to the vessel wall and extravasation of activated T cells and leukocytes into the tumor parenchyma.\(^17\) Recruitment of immune cells to the tumor environment may be an integral part of the adjuvant effect of radiation on the immune response.

In summary, the many effects of ionizing radiation are sensed by the immune system. It is likely that the combination of tumor antigen release, immunogenic cell death, cytokine release, and cell trafficking all contribute to the adjuvant effects of radiation.

**Current resources**

The general decline in funding levels, and the lack of interest in radiation biology within the immunology community has resulted in only a few grants specifically designated for study in this area. The cross discipline nature of research in this area is both advantageous and detrimental. Traditional funding for radiation grants through NIH, ASTRO, RSNA and other radiation focused groups are available. In parallel, a broad pool of immunological grant funding is also possible through the NIH and cancer oriented immune foundations like the Cancer Research Institute. However, the immunologic response to radiation has less general appeal to the immunologist and often when these applications are reviewed by radiation oncologists or radiation biologists they are poorly recognized.

However, a recent effort from NIH is likely to modify the current situation. Under the sponsorship of the Radiation Research Program of the National Cancer Institute, the two days symposium on “Modulation of Tumor Immunity with Hypo-fractionated and special Multi-Fraction Radiation therapy : Basic Mechanisms and Clinical Implications”, on April 4-6, “the meeting enabled 37 oral presentations on this subjects and offered ample opportunities for discussion of the many unsolved issues about combining radiotherapy and immunotherapy. Importantly it enabled communication of the investigators with NIH officers and Industry representatives interested in immune modulation by radiotherapy. Hopefully, it will serve as platform for future exchanges and growth of the field.

The necessary core resources to enable this field of research are also limited. These include immuno-monitoring facilities and cancer centers commitment to support costly immunological studies often associated with immunotherapies. From a preclinical research point of view, immunology experiments looking at complex cellular interactions typically require costly syngeneic animal models and the possibility to selectively irradiate the primary tumor site, while sparing the rest of the animal to enable detection of abscopal effects. Animal irradiators are commercially available, but they require space and operating funding to be functional and accessible to interested investigators.

**Future potential**

While our understanding of the immunomodulatory effects of radiation is in its infancy, its future is promising in terms of the translational impact. Radiation, unlike chemotherapy is anatomically targetable. This makes the use of high dose conformal radiation attractive as a non-invasive localizable immune adjuvant, devoid of significant systemic immunosuppression. Importantly, when combined with the appropriate (Continued on next page)
immunotherapy, radiation has shown the unique capacity to convert the primary tumor into an in situ, individualized vaccine, as proven by rapidly emerging anecdotal clinical reports.18

Future research will focus on understanding how best to potentiate cancer immunotherapy with radiation, and how to optimally select cancer vaccines and immune modulating drugs for combination treatment. At the same time, research to mitigate the inflammatory toxicities of radiation therapy will also significantly affect clinical radiation oncology.

Several new cancer immunotherapy agents have recently been FDA approved and many more appear effective in early trials. Combining radiation with these immunotherapies offers significant clinical promise. Ipilimumab, heads the class of drugs that removes restraints on T cell activation. While radiation may boost the effectiveness of these medications, as seen with Ipilimumab, there is also the risk of developing autoimmunity. Our current understanding of the balance between these outcomes is poor and of major importance. While Ipilimumab targets CTLA-4, a major inhibitor of costimulation, many other costimulatory inhibitors, with more manageable toxicity profiles, such as anti-PD-1 antibodies are under investigation. Of notice, they have shown preliminary efficacy across many cancer types. Research of their use when combined with radiation is ongoing and has the potential to offer more favorable risk/benefit profiles.

In addition to targets of T cell function, the immune system has many interacting cell types with multiple positive and negative signaling pathways. This complexity open the way to a completely new field, to study opportunities of synergy of radiation with cancer immunotherapy.

In parallel, understanding exactly how radiation affects inhibitory immune cells like regulatory T cells may lead to the development of drugs to augment the effects of radiation on T cell recruitment and priming. A primary goal of cancer immunotherapy is to trigger effective T cell responses. Tumor infiltrating T cells, while often specific for cancer antigens, are often non-responsive. This anergic state may relate to chronic stimulation through the T cell receptor (TCR), or lack of costimulatory signals from professional antigen presenting cells. Radiation has shown the ability to overcome these peripheral tolerance mechanisms. In addition to the quantity of activated T cells, the quality of T cell activation may have a dramatic effect on tumor rejection. Activated CD4 T cells can take on several patterns of cytokine secretion and are termed, Th-1, Th2, and Th-17 responses based on this profile. Traditional vaccines use aluminum salts (Alum) as an adjuvant. Alum based vaccines produce Th-2 responses skewed towards humoral immunity which has proven ineffective for cancer immuno-

To activate an effective immune response the involved immune cells must traffic between the tumor and lymph node areas. As described, irradiation provides signals for this trafficking to the tumor site by vascular effects and chemokine secretion. Beyond this we have little understanding of the trafficking effects of radiation. As an example, there is evidence radiation works synergistically with Ipilimumab in the treatment of melanoma brain metastases. It is unclear how radiation improves the effect of Ipilimumab alone. This could be related to radiation break down of the blood brain barrier allowing Ipilimumab or activated T cells into the brain. The recent success of “synthetic biology,” using engineered T cells specifically expressing chimeric T cell receptors, provides a great opportunity to use radiation to target these to solid tumor sites.19 Greater understanding of the effects of radiation on cell trafficking holds potential for improving treatment of some of the most difficult sites of oncologic treatment.

Interestingly, consistent confirmation of preclinical results of abscopal effects has been reported in the clinic. Moreover some of these reports demonstrate successful immune rejection of tumors in the metastatic setting, offering an expansion of the role of mere palliation reserved to radiotherapy.3

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**Needs within the profession**

There are several barriers to effective research in this field. Study of the immune "system" inherently relies on the interactions of many cell types making it difficult to study in vitro, and necessitating animal models. Radiation techniques can be effectively applied to some well-established preclinical immune models. However traditional tumor transplant models in immunocompromised mice are unsuitable, as this inherently defeats the goal of the research. To prevent rejection due to host tumor histo-incompatibility, one must rely on more limited mouse models using syngeneic tumors or genetically engineered autochthonous cancers. This also means that human tumors cannot be used, which makes translating research from preclinical immune models to human trials more challenging. Therefore, the model systems are complex, and substantially more costly in time, effort, and funding. Improved models, with shorter tumor development times, intact immune systems, and the use of human derived tumors are warranted.

Additionally, technical improvement in measuring antigen specific T cell responses in humans is needed. Unfortunately, the best available methods rely on advanced knowledge of the relevant cancer specific-antigens (which are usually unknown) and the use an MHC/tetramer reagent to identify specific T cell responses. These tetraramers work very poorly for CD4+ T cells, a major T cell subtype. Effectively tracking specific T cell responses in patients with tetraramers is not practical, due to technical reasons involving diversity in the major histocompatibility complex (MHC). This barrier severely limits the ability to conduct effective immune monitoring research and to correlate immune parameters with the responses seen in current immunotherapy trials. Alternatives such as ELISpot or cytokine capture assays have their own limitations and have failed to demonstrate significant correlations with outcomes in cancer immunology trials. Much of our current data on serum antibody response assays that have changed little in the past 50 years. Until more specific and effective methods to follow immune responses are identified, cancer immunotherapy trials will use crude methods of assessing the immune response, limiting insightful interpretation of the results. Next generation sequencing techniques may hold promise as a means to track the global T cell repertoire in peripheral blood and follow specific clonal T cell responses. In theory, this approach can track responses to multiple antigens simultaneously, unlike tetraramers. The lack of current effective T cell tracking assays remains a major limitation to this field.

**Time frames to achieve various goals:** Within the next ten years we anticipate significant advances in our understanding of how radiation contributes to an immune rejection of tumors. The technical capabilities and the academic interest to define the optimal doses, fractionation and timing of radiation treatments are in place. Advances in the basic science of immune response to radiation is rapidly progressing.

Genetically “knockout” models are available and the relative contribution of the different components of the immune system can be tested. The role of specific immune cells can be evaluated and determining the cytokines, chemokines and cell surface immune molecules these cells express will be important to design strategies to generating productive radiation immune effects. In the next five to ten years this understanding will be incorporated in the practice of clinical radiation oncology, as several trials are already ongoing combining radiotherapy and immunotherapy in metastatic disease.

**Infrastructure needs:** Although the desire to study the immune effects of radiation is rapidly growing, there are limited resources and expertise in this area. Immune models and study are resource intensive and advancements require capital investments and infrastructure investments in areas such as flow cytometry, small animal facilities, next generation sequencing, and parallel cytokine detection platforms. The integration of “core facilities” at larger academic centers have provided less experienced researchers with access to expertise in immunologic assays and testing that would otherwise be too complex or expensive. This paradigm should be emulated and expanded.

**CHAPTER REFERENCES**


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Development of curricula for the education of trainees in radiation oncology is the responsibility of the Radiation Oncology Residency Review Committee (RO RRC) of the Accreditation Council on Graduate Medical Education (ACGME) and testing those trainees to assure their base of knowledge and skills is the responsibility of the American Board of Radiology (ABR). The ACGME offers general requirements for radiation oncology residency programs regarding education in the biological sciences (see Appendix IV). The ABR offers a “study guide” for radiation and cancer biology on its website (www.theabr.org) to assist trainees preparing for the qualifying (written) examination in the basic sciences but this guide essentially represents a compilation of all identified biology-related topics that might be included on any individual examination (see Appendix V). ASTRO also has a radiation biology study guide subcommittee that has developed and maintains a “study guide” of basic science topics. No attempts are made in any of these outlines to prioritize listed topics, weight by “value,” or to regularly eliminate topics that are diminishing in scientific or clinical relevance.

The ABR has independently taken significant steps forward to facilitate the development of a cadre of clinician-scientists within the profession by creation of the Holman Research Pathway (HRP) for initial ABR certification. The HRP has been highly successful in increasing the likelihood that trainees who have completed the program will pursue academic, research-oriented careers, but as with the primary area of federally-funded research grants (R-01), candidate research projects are investigator-initiated and may lack cohesiveness with the over-arching needs and direction of the specialty. Career research development among the HRP trainees and other, non-HRP clinician-scientists may be significantly hampered by the general lack of post-graduate laboratory commitment within departments, and by the typical requirement that these clinician-scientists supplement their incomes via clinical care activities. Non-physician radiation scientists are not subjected to specific organizational curricular constraints outside of their parent training institutions and as such, form a disparate group that must somehow be developed in a more focused direction to meet the long-term needs of the specialty.

Primary responsibility for education of post-residency radiation oncology practitioners falls to specialty societies such as ASTRO and the Radiological Society of North America (RSNA). These organizations have developed significant programming in the clinical and technical aspects of the specialty but the focus on biological investigation has remained a secondary topic. The Radiation Research Society (RRS) has a Scholars-in-Training (SIT) Program that organizes a one-day workshop before its Annual meeting, and the meeting also has a series of early morning Educational Review lectures. A new RRS initiative is a development fund that aims to support young investigators and junior faculty by providing funding for short-term sabbatical training and pilot/bridge grants. Due to the biological focus of RRS members most of these efforts are directed at radiobiology.

CHAPTER REFERENCES


Recommendations

It was not within the scope of the TF mandate to develop strategies to operationalize the recommendations made, nor to enumerate the policy-making steps necessary to move those recommendations forward although that effort was debated. To move forward with any or all recommendations of the TF will require collaboration between multiple stakeholders, development of strategic plans and budgets, and determination of policy agendas. Attempts to prioritize areas of scientific investigation presume fore-knowledge of research outcomes and the assignment of “value” to specific projects. Operational planning was not within the mandate of the TF and ranking of scientific efforts all of which were felt to be critical to the specialty, was determined to be inappropriate.

These recommendations represent a consensus of the TF members and are not presented in any specific order of priority.

1. The TF believes that the areas of scientific investigation identified represent critical lines of investigation for the radiation oncology enterprise over the next decade, and should be actively pursued by our basic science laboratories. No attempt at prioritization was deemed to be appropriate. This attempt at “trend-spotting” should in no way serve to detract from ongoing research efforts in existing programs or from the value of those efforts.

2. The TF believes that the ABR Holman Research Pathway (HRP) should, if feasible, be expanded, as should other innovative methods of encouraging trainee research projects and careers. The research careers and achievements of HRP and non-HRP clinician scientists will be significantly hampered if opportunities for research-only or research-focused post-doctoral opportunities are not made available and it is incumbent upon leadership of the specialty that these experiences be encouraged and supported.

3. Basic science testing of residents for initial certification by the ABR should be expanded to include the areas of emerging science noted in this report. As a critical element of this expansion, areas of science felt to be more limited in current clinical relevance should be reduced in emphasis. A critical element of any increase in resident research is stable access to funding and infrastructure support necessary to enable successful implementation and completion of resident-developed projects.

4. A coordinated effort to attract PhD level scientists to the radiation research-related areas of scientific endeavor must be established and resources for these scientists to flourish must be identified and secured.

5. The TF believes that in pursuit of these areas of investigation, a coordinated and strategic policy effort should be made within the Federal research funding programs, to increase support for the training, infrastructure, and projects necessary to pursue these endeavors. ASTRO and other interested stakeholders currently pursue policy efforts designed to increase funding for the “general” radiation research enterprise, but in addition, efforts should be actively pursued to improve the potential for funding of specific high-value, high-quality projects and the supporting individual institutional infrastructure necessary to develop or enhance centers of excellence in radiation-related cancer biology and radiation biology. Currently, grant submissions in radiation biology as considered by the TF, are evaluated and scored for funding by the NCI, Center for Scientific Review (CSR), Radiation Therapeutics and Biology (RTB) Study Section. This review section considers applications involving therapeutic interactions of ionizing radiation, radionuclides, electromagnetic radiation, and heat at the molecular, cellular, organ and patient levels. The study section roster includes physicists, imaging experts, and other reviewers.

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not felt to be authoritative in the depth and breadth of radiation biology applications submitted. The nature of these reviews and reviewers is felt to negatively impact the scoring and potential funding of radiation biology applications, and revision of the evaluation system should become a significant ASTRO policy initiative.

6. The TF believes that the current NIH, and other Federal agency methods of reporting funding for radiation-related research significantly impedes efforts at development of cohesive policies and research strategies of interest to the specialty. A methodology by which investigators can self-designate research activities as “radiation research-related”, designate relationship to a radiation oncology department, or other appropriate nomenclature should be encouraged, as should the ability to evaluate funding by this categorization.

7. The TF believes that in support of these recommendations, there should be an intensive effort to strengthen the basic cancer biology/radiation biology curricula of post-graduate training programs to better prepare residents in radiation oncology to understand and expeditiously adapt new scientific discoveries into their clinical practice and to encourage research efforts in these areas of investigation. Because many smaller training programs have limited resources available for education in every aspect of emerging areas of science, it would be worthwhile to consider a broad variety of innovative training pathways, such as on-line courses and centralization of some portions of resident education in the basic sciences. Where possible, trainees committed to basic research should have access to institutional funding to support participation in national and international basic science meetings. Mentoring of trainees committed to basic research must be improved and critical assistance with early career investigators grant submissions must be provided. Especially in smaller training programs, this mentorship and review may be necessary from investigators in other departments or institutions if senior mentorship is not internally available. The TF advises that grant applications from early career investigators should undergo critical and constructive senior mentor review prior to submission. Concurrent with adoption of emerging areas of scientific investigation into training program curricula, the ABR should update its cancer and radiation biology qualifying examination to include these new areas of investigation. Concurrent with increase in the number of potential investigators, the specialty must seek progressive growth in infrastructure and stable funding mechanisms.

8. The TF believes that ASTRO’s Annual Meeting Scientific Program Committee should be encouraged to actively seek to provide podium sessions and courses on the scientific topics identified. Where the Society Annual Meeting is not felt to be an appropriate venue for focused and in-depth consideration of specific topics, development of smaller regional meetings should be considered. The nature of these designated areas of investigation are of such complexity that expansion of innovative opportunities for joint programming between ASTRO and other scientific organizations such as the American Association for Cancer Research and the Radiation Research Society should be considered. Rather than pursuing joint meetings between the various societies, which have been attempted in the past with limited success, efforts should focus on incorporation of individual speakers and/or panels, dealing with highly selected scientific topics, into regional and national meetings. Whenever possible, ASTRO should attempt to include international radiation research investigators in its programming.

9. The 5-year period following completion of residency training is critical in the establishment of research-oriented careers. This transitional period is especially important for ABR HRP trainees and other non-Holman individuals who have already exhibited a commitment to research. Just as trainees in medical oncology or pediatric oncology receive mentored basic science training for 3, 4 or more years at the post-graduate level, the TF believes that opportunities to extend protected time for mentored research training beyond the research-oriented residency or HRP are needed so that current trainees in radiation oncology have the skills, experience, and publication track record to successfully compete with oncologists from other fields when they become independent investigators. The TF recommends that ASTRO investigate expansion of a “bridge fund” program to assist these young investigators during the period before they can establish successful laboratories and attain independent research funding.

10. Radiation oncology is a relatively small specialty with a limited number of committed investigators and finite resources. As such, optimizing the work of individuals and value of resources is critical. The TF believes that a “clearing house” of personnel, projects, and resources should be developed and made available to interested individuals, and that the Society should create a variety of opportunities for research-committed individuals to network.

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11. The TF believes that the tasks enumerated in this report are of a critical concern to the Society and the specialty, such that all stakeholder organizations, including, but not limited to ASTRO, the Society of Chairmen of Academic Radiation Oncology Programs (SCAROP), the Association of Program Directors of Radiation Oncology Programs (ADROP), the Association of Residents in Radiation Oncology (ARRO), the ABR, AACR, and RRS, should convene a high-level “summit” to develop a strategic plan that includes budgets and timelines to operationalize these goals. The recommendations of that “summit” should be incorporated into the strategic plan of the Society and communicated to the leadership of the various funding agencies.

12. It was apparent from TF interviews that the breadth and depth of current activities in cancer biology and radiation research within the radiation oncology enterprise are little known or recognized outside of the profession. Efforts should be made to aggressively and widely “market” the activities of these researchers, especially those early in their careers.

13. The nature and rapidity of scientific progress and discoveries are such that any periodic review carried out at intervals of five or more years runs a significant risk of irrelevance or lack of timeliness. The TF recommends that a “Scientific Advisory Board” be convened and supported by ASTRO, with membership consisting of influential scientists and clinicians from inside the radiation oncology community and from outside that enterprise, and including individuals from outside the USA. This committee should meet periodically to review and update ASTRO scientific programs and recommend proposed policy changes. Committee members must represent a variety of scientific disciplines, career and funding levels, and where possible, should have access to national research funding and development policymakers.
Conclusions

The TF charge from the ASTRO BOD was to focus on the future of radiation biology research in its role of advancement of the clinical specialty of radiation oncology. In its deliberations, the TF made no effort to evaluate the merits of current radiation research centers, investigators or projects, and none of the TF recommendations should be perceived as disparagement of those facilities, personnel, or projects. Translational (Phase I) and Phase II or III clinical investigations, as well as physics and technical research were not considered by the TF, except for considerations of how basic, pre-clinical investigation might impact those endeavors. In its discussions, the TF did consider several inexorable facts that weighed heavily on its ultimate recommendations. These included:

- Radiation oncology is, and will remain, a relatively small specialty with limited resources to support dedicated basic research efforts, but with an inordinate degree of benefit to cancer patients.
- Although the ability to deliver higher and more accurate doses of radiation has advanced the treatment of many cancers, maximizing further improvements in the outcome of cancer patients treated with radiation therapy will likely not depend on technological improvements in dose delivery, but instead, will depend on advances in understanding and utilizing the effect of radiation as a potent modulator of genetic and cellular activity.
- The nature of the radiation research enterprise is such that it will survive and flourish only if its efforts are directed primarily in support of clinical radiation oncology, rather than simply attempting to adapt agents developed by and for medical oncology to radiation-related use. The research and systemic agent needs of clinical radiation oncology are such that government funding efforts will relate more directly to answering broader scientific questions, and pharmaceutical company initiatives will focus on fulfilling more significant commercial implications. The responsibility of developing unique agents that will impact radiation effect will fall primarily on our own laboratories and investigators.

As charged, the TF has made recommendations in support of its findings, but developing operational strategies or tactics, or definition of resources necessary to bring its recommendations to fruition, were beyond the scope of the TF mission. No attempt was made to prioritize areas of scientific investigation or recommendations, but the TF does recommend that these issues be considered concurrently rather than sequentially by ASTRO policy-makers and other stakeholders.

The nature of the radiation research enterprise is such that it will survive and flourish only if its efforts are directed primarily in support of clinical radiation oncology, rather than simply attempting to adapt agents developed by and for medical oncology to radiation-related use.
## APPENDIX I:

ASTRO Cancer Biology/Radiation Biology Task Force Members and Contributors

### Table A.1. Members and Contributors

#### Task Force Members

<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paul E. Wallner</td>
<td>Chair, 21st Century Oncology, LLC and the American Board of Radiology</td>
</tr>
<tr>
<td>Mitchell Anscher</td>
<td>Virginia Commonwealth University</td>
</tr>
<tr>
<td>Christopher A. Barker</td>
<td>Memorial Sloan-Kettering Cancer Center</td>
</tr>
<tr>
<td>Michael Bassetti</td>
<td>University of Wisconsin Carbone Cancer Center</td>
</tr>
<tr>
<td>Robert G. Bristow</td>
<td>Princess Margaret Cancer Center/University of Toronto</td>
</tr>
<tr>
<td>Yong I. Cha</td>
<td>Norton Cancer Institute</td>
</tr>
<tr>
<td>Adam Dicker</td>
<td>Thomas Jefferson University</td>
</tr>
<tr>
<td>Silvia C. Formenti</td>
<td>New York University</td>
</tr>
<tr>
<td>Stephen M. Hahn</td>
<td>University of Pennsylvania</td>
</tr>
<tr>
<td>Tom Hei</td>
<td>Columbia University</td>
</tr>
<tr>
<td>Alec Kimmelman</td>
<td>Dana-Farber Cancer Institute</td>
</tr>
<tr>
<td>David Kirsch</td>
<td>Duke University</td>
</tr>
<tr>
<td>Kevin Kozak</td>
<td>University of Wisconsin</td>
</tr>
<tr>
<td>Theodore S. Lawrence</td>
<td>University of Michigan</td>
</tr>
<tr>
<td>Brian Marples</td>
<td>Oakland University</td>
</tr>
<tr>
<td>William McBride</td>
<td>University of California Los Angeles</td>
</tr>
<tr>
<td>Catherine Park</td>
<td>University of California San Francisco</td>
</tr>
<tr>
<td>Joanne B. Weidhaas</td>
<td>Yale University</td>
</tr>
<tr>
<td>Anthony L. Zietman</td>
<td>Massachusetts General Hospital</td>
</tr>
</tbody>
</table>

#### Consultants

<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kevin Camphausen</td>
<td>Radiation Oncology Branch, National Cancer Institute</td>
</tr>
<tr>
<td>Eric Bernhard</td>
<td>Radiation Research Program, National Cancer Institute</td>
</tr>
<tr>
<td>Michael L. Steinberg</td>
<td>University of California Los Angeles</td>
</tr>
</tbody>
</table>

#### Non-Task Force Section Contributors

<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edward E. Graves</td>
<td>Stanford University</td>
</tr>
<tr>
<td>Ross B. Mikkelsen</td>
<td>Virginia Commonwealth University</td>
</tr>
</tbody>
</table>
Summary of Survey Statistics

- Accounting for duplicates across the mailing lists, a total of 1,690 survey instruments were distributed to individuals on the mailing lists of ASTRO and RRS. Responses were received from 465 (response rate of 28%), of which 395 were felt to be valid and evaluable.
- Because statistical significance is not felt to be relevant in the context of this material, and because many individuals responded only to questions specifically related to their own responsibilities and/or activities, only percent of respondents are reported for specific observations to demonstrate trends.
- Percentages are rounded to the nearest full number.
- All respondents did not answer every question, and some questions were not designed to be answered by all respondents.

1. Demographics

1.1. Primary Work Location (figure A.1)

1.1.1. Primary work location for 85.5% of respondents was identified as the United States.
1.1.2. Respondents worked in 36 states and the District of Columbia, with California being the only state with > 10% of respondents (10.2%).
1.1.3. New York and Pennsylvania both approach California with 9.0% and 8.1% respectively.
1.1.4. Sections of the country, defined for respondents, were generally evenly represented.
1.1.5. Majority (85.5%) of respondents identified their primary work setting as either an academic health center, university system, or cancer center.

1.2. Roles/Age (figure A.2)

1.2.1. The majority of respondents were ROs or cancer biology/radiation biology researchers.
1.2.2. Half of respondents (49.7%) were over the age of 48; 64% were between the ages of 50-80.
1.2.3. Close to half (43%) of respondents indicated that they plan to continue active work after the age of 70.

1.3. Respondent Education (figure A.3)

1.3.1. Most respondents (70%) obtained their graduate degrees in the United States.

FIGURE A.1: Regional Location

- South 28%
- West 18%
- Northeast 26%
- Midwest 27%

FIGURE A.2: Primary Role

- RO: 26%
- CB/RB Researcher: 24%
- CB/RB Instructor: 14%
- RO Resident: 15%

FIGURE A.3: Education

- Masters: 61%
- MD: 58%
- PhD: 9%

(Continued on next page)
2. Research Experience

2.1. More than half (61%) of cancer or radiation biologists (CB/RB) received their training “on the job”

2.2. About half (42%) of respondents currently teach/conduct more than 9 lectures per year

2.3. Close to half (46%) of respondents entered the research workplace less than 15 years ago

3. Research Resources and Funding

3.1. Research professionals spend about one third of their time (31.5%) on research while patient facing professionals spend close to half of their time (45%)

3.2. Research training

3.2.1. Two thirds (65%) of respondents indicated that they had an opportunity to participate in basic research during their radiation oncology training, but only 50% indicated the experience was either “good” or “excellent”

3.2.2. More than half of respondents (57%) of respondents indicated that they believed that greater access to on-line basic science materials would be beneficial to their training

3.2.3. Close to one third (36%) of respondents indicated that they had received any training in grant development, writing, or management.

3.3. Funding activity

3.3.1. More than one quarter (30%) have applied for NCI grants in the last 5 years;

3.4. Funding sources

3.4.1. Of respondents who received funding for research in the preceding 5 years, funding was most often (30%) received from their primary institution
3.5. Laboratory resources

3.5.1. Little more than one quarter (28%) of respondents identified clinical radiation oncology departments as the “residence” of their laboratories.

3.5.2. Lab space is most often shared by faculty within a department.

3.5.3. Researchers indicate additional space would yield more projects, but funding is an issue for most of them (78%).

3.6. Research support

3.6.1. More than half (56%) of respondents indicated that they had experience a decrease in their personal research funding during the preceding 5 years, with a similar decrease noted in total departmental research funding.

3.7. Research efforts

3.7.1. The majority of respondents (64%) identify as clinical trials researchers.

3.7.2. Respondents believe the specialty would benefit from the development of centralized, more focused programs for the following:

| Training of scientists and residents | 8%  |
| Specialty wide incubators for research | 65%  |
**TABLE A.5. Interview Script**

<table>
<thead>
<tr>
<th>Question</th>
</tr>
</thead>
<tbody>
<tr>
<td>Could you describe for me your role in your institution and in the</td>
</tr>
<tr>
<td>greater universe of oncology research and clinical care?</td>
</tr>
<tr>
<td>Do you have any current role or relationship with the radiation oncology</td>
</tr>
<tr>
<td>department in your institution, or with radiation oncology organizations?</td>
</tr>
<tr>
<td>What do you consider to be the current state of radiation oncology</td>
</tr>
<tr>
<td>within the spectrum of cancer care?</td>
</tr>
<tr>
<td>What does the term “radiation biology research” actually mean to you?</td>
</tr>
<tr>
<td>Where do you see radiation research today?</td>
</tr>
<tr>
<td>Where do you think radiation research (biology) should be going in the</td>
</tr>
<tr>
<td>next 10 years?</td>
</tr>
</tbody>
</table>
Accreditation Council on Graduate Medical Education (ACGME) Program Requirements for Graduate Medical Education in Radiation Oncology Eff. 1/1/09

(Reprinted with permission from the Accreditation Council on Graduate Medical Education, March 2013)

IV.A.5.B) MEDICAL KNOWLEDGE

Residents must demonstrate knowledge of established and evolving biomedical, clinical, epidemiological and social behavioral sciences, as well as the application of this knowledge to patient care. Residents:

(1) must have instruction in the basic sciences essential to Radiation Oncology including medical physics and radiation and cancer biology;
American Board of Radiology (ABR)
Study Guide:
Radiation and Cancer Biology

(Intended for guidance in preparation for the ABR qualifying examination (written examination) in Cancer and Radiation Biology)

(Reprinted with permission from the American Board of Radiology, March 2013)

This exam tests your knowledge of the principles of cancer and radiation biology underlying the practice of radiation oncology. Included are questions on:

- basic cancer biology and the molecular biology of cancer
- the response to radiation at the sub-cellular and cellular levels
- the radiation responses of normal and malignant tissues
- radiation carcinogenesis
- hereditary effects as they relate to radiation protection
### TABLE A.6. Categories for CB and RB

#### BRACHYTHERAPY
- Dose rate effects (HDR and LDR)
- Choice of isotopes
- Interstitial and intracavitary use
- Radiolabeled antibodies

#### CANCER
- Cancer as a genetic disease
- Oncogenes
- Tumor suppressor genes
- Telomeric changes in cancer
- Epigenetic changes in cancer (e.g., hypermethylation)
- Multistep nature of carcinogenesis
- Molecular profiling of cancer
- Signaling abnormalities in carcinogenesis
- Effects of signaling abnormalities on radiation responses
- Prognostic and therapeutic significance of tumor characteristics

#### CELL AND TISSUE KINETICS
- Cell cycle
- Measurement of cell cycle parameters by 3H-thymidine
- Measurement by flow cytometry, DNA staining and BrdU
- Cell cycle synchronization techniques and uses
- Effect of cell cycle phase on radiosensitivity
- Cell cycle arrest and redistribution following irradiation
- Cell cycle checkpoints, cyclins, cyclin dependent kinase inhibitors
- Tissue kinetics
  - Growth fraction
  - Cell loss factor
  - Volume doubling times
  - Tpot

#### CELL AND TISSUE SURVIVAL ASSAYS
- In vitro clonogenic assays
  - Effects of dose, dose rate, cell type
- In vivo clonogenic assays
  - Bone marrow stem cell assays, jejunal crypt stem cell assay, skin clones, kidney tubules

#### CHEMOTHERAPEUTIC AGENTS AND RADIATION THERAPY
- Classes of agents
- Mechanisms of action
- The oxygen effect for chemotherapy
- Multiple drug resistance
- Interactions of chemotherapeutic agents with radiation therapy
- Photodynamic therapy
- Gene therapy

#### CHROMOSOME AND CHROMATID DAMAGE
- Assays
- Conventional and FISH
- Dose response relationships
- Use of peripheral blood lymphocytes in in vivo dosimetry
- Stable and unstable chromatid and chromosome aberrations
- Human genetic diseases that affect DNA repair, fragility, and radiosensitivity

#### CLINICALLY RELEVANT NORMAL TISSUE RESPONSES TO RADIATION
- Responses in skin, oral mucosa, oropharyngeal and esophageal mucous membranes, salivary glands, bone marrow, lymphoid tissue bone and cartilage, lung, kidney, testis, eye, central and peripheral nervous tissues
HYPERTHERMIA
- Cellular response to heat
- Heat shock proteins
- Thermotolerance
- Response of tumors and normal tissues to heat
- Combination with radiation therapy

HERITABLE EFFECTS OF RADIATION
- Single gene mutation
- Chromosome aberrations
- Relative vs absolute mutation risk
- Doubling dose
- Heritable effects in humans
- Risk estimates for hereditable effects

INTERACTION OF RADIATION WITH MATTER
- Definition of ionizing radiation and types
- Definition of LET and quality of radiation
- Generation of free radicals
- Direct and indirect action of radiation
- Role of oxygen

LINEAR ENERGY TRANSFER
- RBE defined
- RBE as a function of LET
- Tissue type Oxygen Effect

MECHANISMS OF CELL DEATH
- Apoptotic death
  - Developmental and stress induced
  - Morphological and biochemical features of apoptosis
  - Molecular pathways leading to apoptosis
  - Radiation-induced apoptosis in normal tissues and tumors
  - Necrotic death
  - Morphological, pathological, and biochemical features of necrosis
  - Mitotic death following irradiation
  - Catastrophic vs. apoptotic death
- Cell division postradiation and time of clonogen death
- Radiation-induced senescence

MECHANISMS OF NORMAL TISSUE RADIATION RESPONSES
- Molecular and cellular responses in slowly and rapidly proliferating tissues
  - Cytokines and growth factors
  - Regeneration
  - Remembered dose
  - Functional subunits
- Mechanisms underlying clinical symptoms
  - Latency
  - Inflammatory changes
  - Cell killing
  - Radiation fibrosis
  - Volume effects
- Scoring systems for tissue injury
  - LENT and SOMA

MODELS OF CELL SURVIVAL
- Random nature of cell killing and Poisson statistics
- Comparison of survival of viruses, bacteria, and eukaryotic cells after irradiation
- Single-hit, multi-target models of cell survival
- Two component models
- Linear quadratic model
- Calculations of cell survival with dose

MOLECULAR MECHANISMS OF DNA DAMAGE
- Assays for DNA damage
- Neutral and alkaline elution, pulsed field electrophoresis, comet, plasmid-based assays
- Types of DNA lesions and numbers per cell/Gy
- Multiply damaged sites
- Single lethal hits and accumulated damage (inter- and intratrack)

MOLECULAR MECHANISMS OF DNA REPAIR
- Types of repair
- Repair of base damage, single-strand and double-strand breaks
- Homologous recombination
- Nonhomologous end-joining
MOLECULAR SIGNALING
- Receptor/ligand interactions
- Phosphorylation/dephosphorylation reactions
- Transcriptional activation
- Gene expression profiling and radiation-induced gene expression
- Radiation-induced signals
  - DNA damage response
  - Non-DNA damage response
- Cell survival and death pathways

OXYGEN EFFECT
- Define OER
- Dose and dose per fraction effects
- OER vs LET
- Impact of O2 concentration
- Time scale of oxygen effect
- Mechanisms of oxygen effect

RADIATION CARCINOGENESIS
- Initiation, promotion, progression
- Dose response for radiation-induced cancers
- Importance of age at exposure and time since exposure
- Malignancies in prenatally exposed children
- Second tumors in radiation therapy patients
- Effects of chemotherapy on incidence
- Risk estimates in humans
- Calculations based on risk estimates

RADIATION EFFECTS IN THE DEVELOPING EMBRYO
- Intrauterine death
- Congenital abnormalities and neonatal death
- Microcephaly, mental retardation
- Growth retardation
- Dose, dose rate, and stage in gestation
- Human experience of pregnant women exposed to therapeutic dose

RADIATION PROTECTION
- General philosophy
- Stochastic and deterministic effects
- Relative weighting factors
- Equivalent dose-tissue weighting factor
- Effective dose, committed dose
- Collective exposure dose
- Dose limits for occupational and public exposure
- ICRP and NCRP

RADIOBIOLOGICAL ASPECTS OF ALTERNATIVE DOSE DELIVERY SYSTEMS
- Protons, high LET sources, BNCT
- Stereotactic radiosurgery/radiotherapy, IMRT, IORT
- Dose distributions and dose heterogeneity

RADIOSENSITIZERS, BIOREDUCTIVE DRUGS, RADIOPROTECTORS
- Tumor radiosensitization
- Halogenated pyrimidines, nitroimidazoles
- Hypoxic cell cytotoxins
  - Tirapazamine
- Normal tissue radioprotection
  - Mechanisms of action, sulfhydryl compounds, WR series, dose reduction factor (DRF)
- Biological response modifiers

REPAIR AT THE CELLULAR LEVEL
- Sublethal damage repair
- Potentially lethal damage repair
- Half-time of repair
- Dose rate effects and repair
- Dose fractionation effects

SOLID TUMOR ASSAY SYSTEMS
- Experimental models
- TD50 limiting dilution assay
- Tumor regrowth assay
- TCD50 tumor control assay
- Lung colony assay
- In vitro / in vivo assay
- Spheroid systems
**THERAPEUTIC RATIO**

- Tumor control probability (TCP) curves
  - Calculation of TCP
  - Factors affecting shape and slope of TCP curves
  - Influence of tumor repopulation/regeneration on TCP
- Normal tissue complication probability (NTCP) curves
  - Influence of normal tissue regeneration on responses
  - Response of subclinical disease
  - Causes of treatment failure
  - Factors determining tissue tolerance
  - Normal tissue volume effects
  - Dose-volume histogram analysis
- Effect of adjuvant or combined treatments on therapeutic rationals

**TIME, DOSE, FRACTIONATION**

- The 4 R’s of fractionation
- The radiobiological rational behind dose fractionation
- The effect of tissue type on the response to dose fractionation
- Effect of tissue/tumor types on a/b ratios
- Quantitation of multifraction survival cures
- BED and isoeffect dose calculations

**TOTAL BODY IRRADIATION**

- Prodromal radiation syndrome
- Cerebrovascular syndrome
- Gastrointestinal syndrome
- Hematopoietic syndrome
- Mean lethal dose and dose/time responses
- Immunological effects
- Assessment and treatment of radiation accidents
- Bone marrow transplantation

**TUMOR MICROENVIRONMENT**

- Tumor vasculature
- Angiogenesis
- Hypoxia in tumors
  - Measurement of hypoxia
  - Transient and chronic hypoxia
- Reoxygenation following irradiation
- Relevance of hypoxia in radiation therapy
- Hypoxia as a factor in tumor progression
- Hypoxia-induced signal transduction
- Cellular composition of tumors