Combining precision radiotherapy with molecular targeting and immunomodulatory agents: a guideline by the American Society for Radiation Oncology

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The practice of radiation oncology is primarily based on precise technical delivery of highly conformal, image-guided external beam radiotherapy or brachytherapy. However, systematic research efforts are being made to facilitate individualised radiation dose prescriptions on the basis of gene-expressssion profiles that reflect the radiosensitivity of tumour and normal tissue. This advance in precision radiotherapy should complement those benefits made in precision cancer medicine that use molecularly targeted agents and immunotherapies. The personalisation of cancer therapy, predicated largely on genomic interrogation, is facilitating the selection of therapies that are directed against driver mutations, aberrant cell signalling, tumour microenvironments, and genetic susceptibilities. With the increasing technical power of radiotherapy to safely increase local tumour control for many solid tumours, it is an opportune time to rigorously explore the potential benefits of combining radiotherapy with molecular targeted agents and immunotherapies to increase cancer survival outcomes. This theme provides the basis and foundation for this American Society for Radiation Oncology guideline on combining radiotherapy with molecular targeting and immunotherapy agents.

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Introduction

Precision radiotherapy is delivered as highly conformal, image-guided external beam radiotherapy or brachytherapy. Radiotherapy alone or in combination with chemotherapy or surgery is an important and often curative method of treatment; indeed, the standard of care for many cancers of the head and neck, lung, gastrointestinal tract, urinary and genital organs, musculoskeletal system, skin, female reproductive systems, and central nervous system includes radiotherapy as part of its treatment regimen.

Radiotherapy is an important treatment modality that is given to over 50% of patients with cancer at some time during the course of their disease. Although radiotherapy alone can be curative for early-stage tumours, improvements in locoregional control and overall survival have been realised in combination with surgery or chemotherapy, or both, for many advanced solid tumours. Over the past decade, substantial advances have been made in precision cancer medicine with molecular targeting drugs. Individualised cancer therapy, which is being increasingly predicated on genomic interrogation, is facilitating the selection of molecular targeting agents directed against driver mutations and aberrant intracellular signalling, tumour microenvironments, and genetic susceptibilities on the basis of synthetic lethality. Efforts to individualise radiation doses on the basis of gene-expressssion profiles that reflect tumour and normal tissue radiosensitivity are just beginning to emerge.[1] Targeted agents could also impact cellular damage and repair pathways, thereby altering the dose-response patterns of radiotherapy. Despite the potential for increased survival rates when pairing molecular targeting with precision radiotherapy, most clinical trials that assess molecular targeting agents are in non-curative, metastatic patient populations. Treatment of these patients with locoregional and metastatic recurrences often selects for aggressive and resistant clones that result in multiple genomic redundancies, which then precludes the successful targeting of a single pathway. Therefore, the probability of getting a so-called genetic match between a targeted drug and a specific mutation within this specific patient population can be very low (5–10%).[2]

An alternative approach of targeting tumours early in their natural history is to combine precision radiotherapy with molecular agents to augment local tumour control or ablate micrometastatic or oligometastatic disease, or both. This approach has the potential to increase absolute survival for patients with cancer. However, a major clinical challenge is to establish the best methodology to develop and implement precision radiotherapy approaches.

In recognition of the substantial advances taking place in combining precision radiotherapy with molecular targeting or immunotherapy, the American Society for Radiation Oncology (ASTRO) Board of Directors commissioned a task group to form a position statement to assess the status and future directions of this rapidly advancing field. RGB and PMH were appointed to direct this effort and assembled an expert panel to contribute to this report. Panel 1 shows some of the areas that have been selected that show the most promise for developing therapies that combine precision radiotherapy and molecular targeted agents.

Herein, we assess preclinical and clinical approaches combining precision radiotherapy with molecular targeting agents to increase tumour control (local and systemic) or decrease toxic effects to normal tissues, or both. The objective of this report is to improve future outcomes for patients with cancer in a curative or palliative care setting.

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Principles of combined radiotherapy and systemic therapy

The advent of molecular targeted agents offers opportunities to improve conventional chemoradiotherapy or to replace chemotherapeutic agents as standard of care in combined treatments in a concurrent, neoadjuvant, or adjuvant setting to radiotherapy. The basis of combining radiotherapy with chemotherapy was defined by Steel and colleagues[3] and Bristow and colleagues,[4] including spatial cooperation with toxicity independence (ie, radiotherapy acts locoregionally and chemotherapy acts against distant micrometastases without toxic interaction between the two), normal tissue protection, and varying degrees of additive tumour cell death.[5]

Molecular targeted drugs can be of use to sensitise tumour cells to radiotherapy (figure 1). EGFR signalling is one example among many in which molecular targeted agents combined with chemoradiation uses tumour-specific alterations. Opportunities exist in the PI3K/AKT/mTOR, VEGF, c-MET, c-KIT, JAK- STAT, anaplastic lymphoma kinase (ALK), and SDF1 or CXCL12 signalling pathways, as well as in cell-cycle checkpoint defects, PARP signalling, hormone signalling, immune checkpoint signalling, and other pathways (table 1). To realise the potential of these opportunities, a thorough investigation of the sequencing and timing of drug delivery with chemoradiotherapy is needed to identify and capitalise on biological cooperation mechanisms.[6,7]

Preclinical models can be used to test the interactions in novel combinations of drugs with radiotherapy since they can explore the effect of agent sequencing on efficacy, and could provide valuable preliminary insights into potential toxic effects. Agents should be tested in vitro, alone and in combination, so that parameters, such as drug concentration and timing for activity, can be established. Along with the knowledge of the toxicity and half-life of the drug in an experimental tumour-bearing animal system, this information can determine the efficacy of these strategies in vivo. Another parameter that should be considered is that the optimal scheduling of the agent with radiotherapy should be done under the same conditions as normal tissues in the irradiated field.

Not all radiosensitisers identified in the preclinical setting will favourably affect outcomes in a clinical trial setting. A series of logically designed hypoxic cell sensitisers showed strong preclinical effects in combination with radiotherapy but little benefit in late-phase clinical trials.[8] Conversely, although a combination of anti-EGFR monoclonal antibody inhibitors with radiotherapy showed modest effects in cell cultures, they showed great effects in animal models and in local tumour control.[9,10] This combination translated into an overall survival advantage for patients with cancer of the head and neck in a phase 3 clinical trial.[11] Careful and systematic study of drug–radiation interaction parameters will assist, but of course not guarantee, the fidelity of translation from preclinical to clinical trial results.

In assessing a targeted agent with radiotherapy, several key questions should be addressed: do the tumour cells express the molecule to be targeted? Is the tumour target functional, and expressed at a clinically relevant level? Further considerations should include a bioassay for the target or an assessment of its downstream effects so that efficacy of the targeting strategy can be monitored. Ideally, the radiation dose used should be specific to the particular cancer being studied. Assessing the appropriate timing of when to administer radiotherapy with targeted therapy is also imperative. For the benefit of future patients with cancer, specifically testing these parameters in clinical trials of radiotherapy with molecular targeting drugs would be highly beneficial and would benefit from systematic collaboration between industry and academic partners.[12,13]

Panel 1: Promising developments in combining precision radiotherapy with molecular targeting agents

Radiotherapy is a curative treatment for many solid tumours and increasing locoregional control could increase the proportion of patients cured by initial therapy.

Radiotherapy is commonly offered at an early stage of tumour progression and so could be a preferred treatment to combine with molecular targeted drugs when genetic instability is less prevalent.

Selected patients presenting with small numbers of oligometastases could be cured with precision radiotherapy to the primary metastases and macrometastases by use of stereotactic ablative techniques combined with targeted systemic treatments.

Acquired resistance to the concomitant use of molecular targeting agents could be reduced when tumours undergo primary clonogenic cell death by use of precision radiotherapy.

The therapeutic ratio of curative treatment can be modified by developing tumour radiosensitisers (and normal tissue radioprotectors), increasing the range of options for combined therapy treatments.

Rigorous preclinical model systems (eg, primary patient-derived xenografts and genetically engineered mouse models) can be valuable to inform clinical trial designs by use of molecular targeted–radiotherapy regimens with associated biomarkers of on-target efficacy.

Reliable standard operating procedures for testing combinations of drugs and radiotherapy in the preclinical setting (including trackable radiation dosimetry based on the National Institute of Standards and Technology guidelines and clinically relevant radiotherapy and drug dosing) will be valuable to select the agents that are most likely to provide meaningful improvements in clinical outcome.

Phase 1 and 2 radiotherapy clinical trials can enable response assessment during treatment, along with companion biological assays or functional imaging studies (PET, MRI, CT), or both, that inform drug pharmacodynamics and mode of action. Responder and non-responder signatures can be developed for predictive assays.

Palliative radiotherapy is routinely given to patients with cancer worldwide and affords important clinical trial endpoint opportunities (eg, quality of life, biomarker assessment) when given in combination with molecular targeted agents.

Preclinical models testing targeted agents with radiotherapy

Human tumour xenograft models implanted into immune-deficient mice are a mainstay of preclinical

testing (table 2). Because of the ease of transplantation, treatment, and follow-up, heterotopic transplantations (tumour transplants into areas that are not the organ of origin) are often the preferred site for initial screening, local tumour control experiments, and studies focused on local tumour responses without considering distant metastases. If the focus is only on local tumour responses not distant metastases, orthotopic tumours (transplanted back into the organ of origin) have the advantage of being within their normal tissue microenvironment and are likely to have more realistic invasive and metastatic behaviour than heterotopic transplants.[14] Furthermore, for tumours such as those of the brain—ie, from areas that have limitations in drug delivery that can restrict treatment efficacy—use of orthotopic models are essential before starting a clinical assessment of a novel treatment regimen. A limitation of all xenograft models is that the use of immunocompromised animals restricts the researcher's ability to investigate the effect of irradiation on interactions with the immune system. Additionally, since the tumour stroma is murine, these models might not be best suited for assessing therapies that target the tumour microenvironment.[15]

Genetically engineered mouse models, which develop spontaneous autochthonous tumours, are interesting models for preclinical studies with or without radiotherapy.[16,17] These models can recapitulate mutational and oncoprotein expression patterns of human tumours while also facilitating the interrogation of the effects of molecular targeted agents on the intact tumour microenvironment and immune system.[17] Additionally, sophisticated animal studies of radiotherapy are possible in models with orthotopically transplanted tumours with the advent of technologies that integrate treatment planning, imaging, and radiotherapy delivery.[18–21] State of the art genetically engineered mouse models, developments in patient-derived xenografts, and technological advances in preclinical radiotherapy and non-invasive imaging have set the groundwork for so-called co-clinical trials, which use preclinical models to faithfully replicate the mutational events observed in human cancers, and to have preclinical studies that parallel ongoing human phase 1/2 clinical trials.[22–24] Mouse models that use established syngeneic murine tumour lines in immunocompetent hosts also provide a powerful experimental system to study cancer therapies in the context of a functional immune system.

Reliable standard operating procedures for testing combinations of radiotherapy and drugs in the preclinical setting need to be established to develop strong preclinical data and to identify compounds that are most likely to provide meaningful improvements in clinical outcomes. The US National Institutes of Health requests for application specifically seek this type of standardisation for studies of radiotherapy and drug combinations that could enable centres of excellence to emerge and provide important foundations of quality control and assurance for improved radiotherapy and drug development.

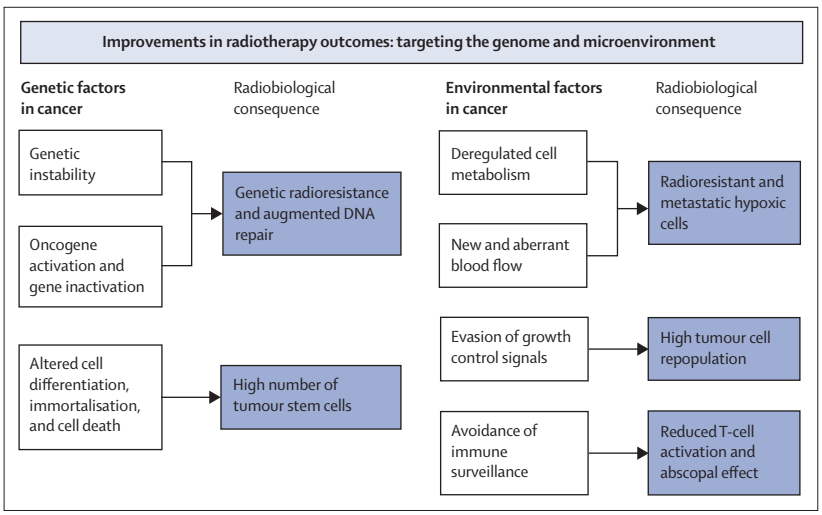

Figure 1: **Genetic and microenvironment cancer factors and their radiobiological consequences**
Cancer cells have a number of inherent processes that cause progression and aggression (so-called hallmarks of cancer). Local tumour control following precision radiotherapy can be compromised by several genetic or microenvironmental biological factors. When correctly combined with precision radiotherapy, molecular targeted agents that target these hallmarks of cancer can improve local control across several tumour types.

Clinical development of targeted therapies with radiotherapy

For precision medicine approaches to be realised, biomarker development is important. Biomarker-driven clinical trials have the potential to be more efficient than they are to date,[25] with the magnitude of efficiency to be gained a result of the size of the biomarker-positive population and the strength of effect in the biomarker-negative population.[26] Phase 1 studies are primarily designed to assess the safety and toxicity of new treatment approaches, but also provide an opportunity to interrogate candidate biomarkers, whereas phase 2 studies are designed to develop data supporting the efficacy of the new treatment combination and understand what subpopulations might specifically benefit from this treatment. The choice of whether to include only biomarker-positive patients or all patients into a clinical trial should be guided by the strength of available evidence regarding a biomarker-effect link, and whether additional data are needed from the trial to inform as to the best treatment options. Alternatively, platform trials have been adopted using master protocols that specify standards and procedures for eligibility and biomarker screening whilst allowing several simultaneous experimental groups with one common control group.[27–29]

As with endpoints, biomarkers can be incorporated into clinical trials in many ways. Including only biomarker-positive patients will provide information only for that group, with no information generated about the predictive capability of the biomarker. This approach might be preferred if strong mechanistic evidence or previous clinical data exist for a biomarker with a well validated assay, particularly in small biomarker

	Drug examples	Potential benefit of treatment	Trial status	Challenges
Intracellular signalling				
EGFR	Cetuximab, panitumumab, gefitinib, erlotinib	Targets tumour cell radioresistance	Phase 2–3 with radiotherapy–chemotherapy combination or radiotherapy alone	Tracking drug action on targets in situ
mTOR	Everolimus, temsirolimus	Targets tumour cell radioresistance	Phase 1/2	Defining additional benefits over combined chemotherapy–radiotherapy
AKT	Nelfinavir	Targets tumour cell radioresistance	Phase 1/2	Needs biomarker-driven trials using functional signalling assays
Cancer metabolism or hypoxia				
Tumour hypoxia	Tirapazamine evofosfamide	Targets tumour cell radioresistance and metastasis	Phase 3	Toxic effects with radiotherapy in head, eyes, ears, nose, and throat (tirapazamine)
Tumour hypoxia	Nimorazole	Targets tumour cell radioresistance and metastasis	Phase 3	Needs biomarker-driven trials
Tumour hypoxia	Metformin	Targets tumour cell radioresistance and metastasis	Phase 2–3	Needs standardisation of hypoxia assays (PET, in situ)
DNA repair and genetic instability				
PARP	Olaparib, veliparib, iniparib	Target tumour radioresistance and use of synthetic lethality	Phase 1	Toxic effects when given concurrently with radiotherapy
ATR	Selumetinib	Target tumour radioresistance and use of synthetic lethality	Phase 1	Selecting patients with appropriate and functional DNA repair mutations in tumours
Immunotherapy				
PD-1 and PD-L1	Pembrolizumab, nivolumab, atezolizumab, avelumab, durvalumab	Augment immune T-cell response and use of abscopal effect	Phase 1–3	Establish tumour sites and biomarkers that are predictive of immune response, targeting low mutation-burden or poorly immunogenic tumours
CTLA-4	Ipilimumab, tremelimumab	Augment immune T-cell response and use of abscopal effect	Phase 1–3	Biomarker-driven trials, targeting low mutation-burden or poor immunogenic tumours
Cytokines	Interleukin-2, interferon, granulocyte-macrophage colony-stimulating factor, and transforming growth factor-β antagonists	Activate effector immune cells and use of abscopal effect	Phase 1–3	Toxic effects when given systemically
Other	Anti-OX40 (TNFRDF4) antibodies, anti-GITR (ENFSF18) antibodies, and TLR-7 and TLR-4 agonists	Augment abscopal effect	Phase 1–2	Integrating with established immunotherapy, managing potentially additive toxic effects
Radioprotection				
Reactive oxygen species	Amisfostine	Protect normal tissues from radiotherapy damage	Phase 2–3	Prove decreased toxic effects and increased therapeutic window
Reactive oxygen species	GC4419	Protect normal tissues from radiotherapy damage	Phase 2–3	Prove decreased toxic effects and increased therapeutic window

AKT=protein kinase B. PARP=poly(ADP-ribose) polymerases. ATR=ataxia telangiectasia-mutated. PD-1=programmed cell death protein-1. PD-L1=programmed cell death protein ligand 1. CTLA-4=cytotoxic T-lymphocyte associated protein 4. TNF=tumour necrosis factor. TLR=toll-like receptor.

Table 1: **Targetable processes for molecular targeted drugs**

populations. Alternatively, if the link between the biomarker and the effect have only been hypothesised on the basis of complex pathway interactions, or if the assays have not been well developed, enrolling both biomarker-positive and biomarker-negative populations might provide more meaningful data than if only one subpopulation was enrolled.

Although identifying small, biomarker-defined responder subpopulations might have its benefits, screening these patients can be inefficient if no plan is in place for the biomarker-negative group. For this reason, platform trials for trials of targeted drugs might be of use. Experimental groups could be added and dropped during the course of the trial without changing the whole infrastructure of the trial.

There are several examples of platform trials with various designs. The NCI-MATCH trial[27] is an early-stage, signal-finding basket study that combines a master protocol for enrolling and genotyping patients with a variety of tumours on the basis of a custom gene-sequencing panel containing 143 genes. This information is then used to assign patients to different

	Advantages	Disadvantages	Preferential use
Experimental human xenograft in immunocompromised animals			
Subcutaneous	Human origin; stable features and fast growth because of selection; ease of transplantation, treatment, and follow-up; applicability of very high radiation doses; long-term follow-up for local tumour control (not limited by distant metastases)	Genetic drift due to selection process during long-term passaging; artificial tumour to normal tissue interactions; immunodeficient host	Screening experiments; large-scale experiments; local tumour control experiments
Orthotopic	Human origin; stable features and fast growth because of selection; tumour growth within normal tissue environment of the original tumour; natural behaviour in terms of invasiveness and metastasis	Genetic drift due to selection process during long-term passaging; requires researchers with a high level of experience for transplantation and follow-up; imaging for response evaluation and follow-up is necessary; radiation dose is limited by surrounding normal tissues; immunodeficient host; time consuming	Experiments that require the tumour to have natural invasiveness or metastasis potential, or both; agents that target the tumour microenvironment
Primary tumour xenografts in immunocompromised animals	Features are close to the original tumour	Difficult to initally establish; lower take rates than for experimental xenografts; immunodeficient host	Preclinical or co-clinical trials—eg, biomarker assessment or new treatments in comparison with patient (donor) treatment
Genetically engineered mouse models	Normal immune system of the host animal; natural and normal tissue environment	Murine tumour; imaging for response evaluation and follow-up is necessary in most cases; radiation dose is limited by the surrounding normal tissues	Experiments that require specific molecular features; co-clinical trials
Immunocompetent rodents for normal tissue experiments	Biology of normal tissues and reactions are similar to humans; many acute and late normal tissue endpoints can be established	Often somewhat more resistant than human-derived xenographs	Comparative experiments on normal tissues—ie, standard versus new treatment

Table 2: **Experimental animal models for combined radiotherapy and molecular targeted agents**

groups testing different targeted agents, with the proportion of patients achieving a response as the primary endpoint. By contrast, the Lung-MAP trial[28] is a phase 2–3 study that genotyped patients before assigning them to one of five randomised studies, in which each study used standard of care as the control group, and in which progression-free survival and overall survival were used as endpoints for all five studies. In both of these examples, a prespecified assignment algorithm was used to identify the right treatment for each group. Another way to assign patients to a treatment groups is by use of data that is gathered during a trial to identify potential biomarker therapeutic links. This Bayesian, adaptively randomised approach was the foundation of the I-SPY 2 breast cancer trial.[29] Biomarker group and treatment effect hypotheses are escalated from this trial for further phase 3 testing when the predicted probability of success of the treatment is high. Two trials that are in devlopment and use a similar approach to the I-SPY 2 trial are the INSIGhT (NCT02977780) and AGILE (NCT02158572) trials, for experimental drugs in combination with radiotherapy for glioblastoma.

Estimates of toxicity must also be considered throughout clinical trial development. Toxic effects in phase 3 trials tend to be smaller than those seen in phase 2 trials, possibly because of under-reporting in larger trials, inclusion of different patient populations in the phase 3 setting, or because of statistical chance. For example, the phase 2 trial of temozolomide plus radiotherapy, followed by temozolomide alone for glioblastoma reported grade 3–4 haematological toxic effects in over 70% of patients,[30] whereas the phase 3 study[31] of the same therapeutic combination had grade 3–4 toxicities in only 7% of patients. These results suggest that the level of toxic effects that should be used as the historical control should be taken from the phase 2 study of the backbone regimen to which the investigational agent will be added. Likewise, investigators should report the number of patients that could not finish the standard of care portion of the treatment, or those who required a substantial delay or break in radiotherapy treatment when treated with a new combination, since these deviations could substantially impair the efficacy of therapy. Often, the effect of such deviations is assessed as part of a previously planned interim analysis. A valuable component of phase 2 studies is tumour pharmacodynamics and assessment of target modulation. This information can correlate with overall outcomes if the drug was successfully on target, if the patient had a better response, or if the target inhibition was independent of patient response.[32]

After completion of a successful phase 2 study that shows an efficacy signal with an acceptable toxicity profile, the population included in the phase 3 trial might be restricted to a biomarker-defined subpopulation identified in earlier developmental stages. When assessing the outcomes of such studies, survival, toxicity, and the cost of the novel combination must be considered.[33] Phase 3 studies are also useful for verifying the usefulness of biomarkers, identifying important

prognostic or predictive markers, and detecting drug–drug interactions in large populations. The future development of novel clinical trial designs, particularly using platform structures, will be crucial to maximising multiplex biomarker assessments and gaining efficiencies from the use of common control groups.

Precision radiotherapy and targeted agents in metastatic disease

The frequent indication for radiotherapy to treat metastatic disease identifies a large patient population that could benefit from the addition of molecular targeted agents. Combined therapy in this clinical setting also provides a foundation for understanding interactions of disease-specific factors, such as tumour-site origin and genetic subtype, with combined therapeutic approaches. Additionally, palliative radiotherapy is frequently given with high dose per fraction regimens, providing unique insights into the effect of targeted agents on improving the therapeutic ratio of hypofractionated radiotherapy. For instance, both prospective and retrospective studies have shown increased local control when combined with hypofractionated radiotherapy for multiple different tyrosine-kinase inhibitors.[34–36]

Although the addition of a drug with known tumour efficacy to standard radiotherapy regimens to improve local control might seem straightforward, the overall effect of intensifying treatment might also increase side effects, and therefore the routine use of combination regimens should be avoided. Surprisingly, early-phase clinical trials for metastatic disease that assess the potential side-effects of combination therapies are uncommon, and thus remain an attractive area of clinical research. Of the studies reported, however, precision radiotherapy given by use of stereotactic techniques appears to not increase side-effects,[36–38] whereas targeted agents with large volume fractionated radiotherapy could confer risks of increased side-effects, and therefore should be undertaken with caution.[39–42]

Several potential patient populations exist for studying the effects of targeted drugs in combination with radiotherapy. Patients with metastatic disease of the brain or spine with a high risk of progression after radiotherapy could benefit from increased local control and a reduction of neurological symptoms associated with disease progression. Additionally, patients with oligometastatic disease could potentially benefit more from combinations of systemic targeted agents with precision radiotherapy than patients with non-oligometastatic disease since the control of gross disease could lead to extended survival benefits.[34,35,43,44] Clinical data suggest that precision radiotherapy with moderate fraction sizes should provide an effective way to combine radiotherapy with molecular targeted drugs, and this hypothesis deserves urgent testing in prospective trials. Such studies should prospectively assess all drug and radiotherapy target sites for increased risk of toxic effects when combining radiotherapy with immune and targeted therapies.

Radioprotection targeting to improve the therapeutic ratio

The goal of using a drug in combination with radiotherapy is to widen the therapeutic ratio (eg, increase tumour cell killing while maintaining or dcreasing normal tissue toxicity). This goal can be accomplished by sensitising the tumour to radiotherapy relative to normal tissues or by protecting the normal tissue from radiotherapy relative to the tumour. Drugs developed as radiosensitisers for cancer should also be assessed in preclinical studies for their effect on normal tissue injury from radiotherapy. The experimental design and endpoint of such studies should be chosen dependent on the mechanism of action of the combined treatment and on the intended irradiation site in patients. To judge the therapeutic window of a treatment, the effect of combining a drug with radiotherapy on the normal tissue dose can be compared with how the combination improves local tumour control.

As ionising radiotherapy induces DNA double-strand breaks, the serine-threonine protein kinase is activated, which orchestrates the activation of a number of downstream pathways including the activation of the cellular tumour antigen p53.[45] In organs in which p53 causes apoptosis, such as the bone marrow and the intestine, blocking p53 or the p53 proapoptotic transcriptional targets could prevent radiotherapy injury.[46–48] Thus, inhibitors of p53 have been proposed as an approach for radioprotection,[49] which could be particularly useful in the setting of radiotherapy for p53-mutant tumours wherein the inhibitor should not affect the response of the tumour cell to radiotherapy. Similarly, preclinical studies suggest that inhibitors of serine-threonine protein kinases and related PI3K family proteins could preferentially sensitise tumours to radiotherapy injury relative to some normal tissues without high levels of cell proliferation.[50–52] Additionally, a study with mice has shown that p53 can be temporarily blocked during total body irradiation to ameliorate acute toxic effects of radiotherapy without increasing radiation-induced cancer.[53]

Although typically thought of as hypoxia-activated proteins, hypoxia-inducible factors (HIFs) can also be activated by radiotherapy. A study[54] using genetically engineered mouse models has shown that the activation of HIF-2α (regulated by prolyl hydroxylase domain-containing proteins) within the gastrointestinal epithelial cells protects mice from radiotherapy injury to the gastrointestinal tract. A pharmacological blockade of prolyl hydroxylase domain-containing proteins with dimethyloxalylglycine prevents gastrointestinal injury before and after radiotherapy. Applications of prolyl hydroxylase domain-containing protein inhibitors or other pharmacological approaches to increase HIF-2α for radioprotection should be explored further as a basis to

widen the therapeutic ratio during the treatment of gastrointestinal cancers.

Combining radiotherapy and immunotherapy

For decades, the field of cancer immunotherapy has explored strategies to engage a patient's own immune system to recognise and eliminate tumours. Historically, the toxic effects of these treatments have restricted their clinical utility. More selective molecular targeted immunotherapies are showing dramatic clinical benefit. Most notable of these new therapies are monoclonal antibodies that block T-cell checkpoint receptors, such as cytotoxic T-lymphocyte antigen-4 (CTLA-4) or the programmed cell death-1 (PD-1) receptor.

Because of the systemic nature of the adaptive immune system, induction of such a response at one tumour site can trigger an antitumour immune response throughout the body. This response raises the possibility that further augmentation of a local antitumour immune response could induce systemic responses. Radiotherapy could locally interact with the immune system by triggering local production of inflammatory cytokines, release of tumour-specific antigens, phenotypic changes in tumour-cell expression of immune-susceptibility markers, vascular effects that enhance immune surveillance, and local eradication of suppressive immune-cell lineages that promote tumour tolerance.[55] Preclinical studies have shown that antigens from random protein mutations are among the most specific and immunogenic tumour antigens recognised by T cells.[56,57] By modulating tumour immune tolerance at a targeted site of disease, and augmenting the accessibility of such antigens to immune recognition, immunotherapy with local radiotherapy could be a method of in-situ tumour vaccination. Such a method might generate a foundation for enhanced presentation of tumour-specific antigens that could stimulate and diversify the systemic antitumour immune response. If proven effective, such approaches could transform radiotherapy from a predominantly locoregional treatment to a crucial component of systemic immunotherapy.

Early preclinical studies investigating combinations of radiotherapy and immunotherapies indicate cooperative interactions with anti-CTLA-4,[58,59] and anti-PD-1 antibodies.[60,61] A subsequent study[61] has shown that such combinations can induce endogenous antigen-specific T-cell and B-cell immune responses in murine tumour models; these responses are associated with enhanced antigen cross-presentation in the draining lymph nodes and increased T-cell infiltration into tumours.[61] Additional reports indicate that the PD-1 ligand, PD-L1, could be upregulated in the tumour microenvironment following radiotherapy, which could result from an extrinsic effect of local cytokine release[62] or through an intrinsic p53-mediated mechanism.[63] Consistent with these reports, some murine tumour models that have not responded to combined treatment regimen of radiation and anti-CTLA-4 antibodies have shown robust radiation-induced upregulation of PD-L1, such that the blockage of PD-1 induces an effective antitumour immune response.[64] Given the multiple cellular and molecular regulators of tumour immune tolerance, future pre-clinical studies can be anticipated to explore the effect of radiotherapy on novel combinations of molecular targeted immunotherapies, of which such strategies are already starting to show promise.[65,66]

Early-phase clinical studies investigating combinations of local radiotherapy with broad-spectrum immune stimulants and T-cell checkpoint blockades appear safe, and suggest an antitumour immune response.[64,67,68] Preclinical and early-phase clinical studies are exploring how radiotherapy dose, fractionation, and timing could influence its interaction with tumour immunotherapy.[66,69] Preclinical studies, although crucial in beginning to address these challenges, could be limited by the fact that differences in animal tumour growth and metabolism might negatively affect the translational relevance of treatment sequencing studies. Early-phase clinical studies are now beginning to explore these parameters in the context of immunotherapies,[70] and such studies will be essential to facilitate appropriate and rational design of advanced-phase clinical trials. Indeed, a 2017 study[71] has suggested that radiotherapy improves the activity of pembrolizumab in patients with advanced non-small-cell lung cancer with a clinically acceptable safety profile. These and other data are required to corroborate preclinical findings about the interaction between radiotherapy and immune checkpoint inhibition in which continued vigilance will be essential to monitor the potential for overlapping toxic effects.

In-situ tumour markers

No biomarkers exist in clinical practice to guide the selection of combinations of radiotherapy with targeted agents. Therefore, optimised preclinical models and a more comprehensive understanding of cancer biology are needed. Extensive data from so-called omics studies support the clinical observation that extensive tumour heterogeneity affects treatment response. This heterogeneity is an obstacle for clinical research that must be overcome to successfully develop novel combination regimens of radiotherapy with immunotherapy or targeted agents. Genomic biomarkers of the effects of radiosensitising drugs could consist of a set of biomarkers or reflect individual genetic alterations. Sets of markers include gene-expression signatures, which could be of use to identify radioresistant cancers to direct the use of radiosensitisers against these tumours.[72–74] Individual markers might include recurrent oncogenic driver mutations, which are increasingly assessed in routine clinical practice, or passenger mutations, which do not affect cell growth or survival in the absence of radiotherapy but become important determinants of survival once cells are damaged by radiotherapy.[75]

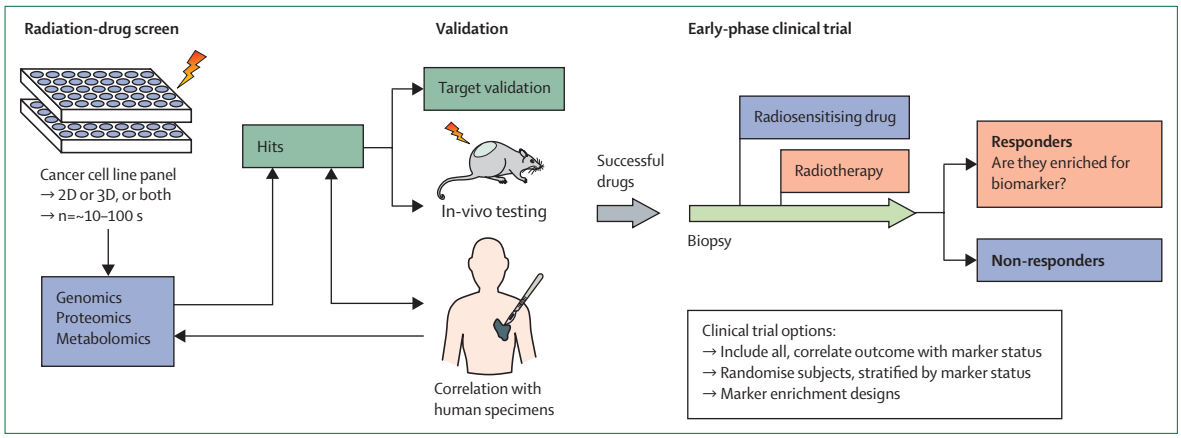

Figure 2: **Methods to identify tumour biomarkers and their treatment effects**
Sophisticated approaches using preclinical models and pre-set, sensitiser enhancement criteria can identify tumour biomarkers (genomic, proteomic, or metabolomic depending on the assay) to predict the effect of molecular targeted agents in combination with radiotherapy. Initial assays should reflect the biology behind factors that influence cellular response to radiotherapy, as outlined in figure 1 (eg, number of tumour clonogens, accelerated repopulation, DNA double-strand break repair, and hypoxia). Validation models should address aspects of both intratumoural and intertumoural heterogeneity in vivo as a way to design combined-modality clinical trials with high-content biomarkers that both confirm on-target effects and increased efficacy compared with radiotherapy alone. Successful testing of such biomarkers in clinical trials involving radiotherapy and targeted agents will hopefully advance the field of precision radiotherapy. Lightning bolt signifies radiation.

The study of functional biomarkers that are of relevance to radiation oncology is of continued interest. In particular, the subnuclear accumulation (ie, foci) of proteins, such as γ-H2AX, as part of the DNA damage response has been used as a surrogate of treatment sensitivity. Preclinical data, for example, have shown the predictive potential of γ-H2AX foci by correlating foci in ex-vivo treated tumour tissues with clinically relevant endpoints, such as tumour control probability.[76] The advantage of using foci as functional biomarkers in patient-derived tumour specimens is that they provide a global measurement of DNA repair function without needing to know the identities of all the components. Thus, foci assays have been hypothesised to give functional insights that could complement or even supersede genomic information.[77] However, additional work is needed to understand whether foci responses in tumour biopsy samples are representative, to standardise assays, and to ascertain the feasibility of fresh tumour tissue collections in clinical practice.

A sophisticated approach is needed to identify predictive local tumour biomarkers of radiotherapy–drug effects. These efforts should include an approximation of clinically relevant intertumoral heterogeneity and the use of integrated tumour profiling. These modern omic approaches should be designed to reflect the biology of established factors that influence response to fractionated radiotherapy. Additionally, patient registries that combine clinical outcomes data with genomic profiling information would be very helpful for biomarker discovery. Successful testing of such biomarkers in clinical trials involving radiotherapy and targeted agents will fundamentally advance the field of precision radiation medicine.

Systemic tumour markers

Although the molecular properties of tumours can reveal aspects of their clinical behaviour and could predict their response to treatment, two important limitations of in-situ tumour markers should be acknowledged. First, they are restricted to a static snapshot of the tumour in both time and space, precluding any analysis of tumour heterogeneity or molecular changes following therapy. Second, patients who have had radiotherapy rarely have large amounts of tissue sampled. These shortcomings could be addressed by the use of systemic biomarkers that are derived from bodily fluids or that are captured as medical images. In the context of preclinical and clinical investigations, systemic biomarkers could provide a four-dimensional assessment of treatment response, leading to more rational combinations of radiotherapy with molecular targeted agents than exist to date (figure 2).

By accessing genetic changes that are not present in other tissues, circulating tumour cells and circulating tumour-derived DNA provide a non-invasive means of tumour genotyping that could be repeated over the course of treatment. Numerous technical platforms exist for the initial isolation and enumeration of circulating tumour cells from peripheral blood.[78] Once isolated, additional molecular analyses can be done to identify predictive features,[79,80] similar to those that would be done on primary tumour tissue samples. Collection, processing, and storage of blood products for downstream analyses are more straightforward for circulating tumour-derived DNA than they are for circulating tumour cells. Similar to circulating tumour cells, numerous technical platforms are in development for the analysis of circulating tumour-derived DNA.[78,81]

The potential predictive value of circulating tumour-derived DNA comes from the identification of mutations that confer resistance to targeted therapies.[82–85] Circulating tumour cells and circulating tumour-derived DNA are poised to transform the way radiotherapy is delivered by enabling real-time monitoring of tumour genotypes and molecular features, and by replacing invasive procedures with blood-based assays. To date, no large-scale longitudinal studies have been done that assess changes in circulating tumour-derived DNA and circulating tumour cells as a biomarker of response in curative radiotherapy regimens. Rapid technological innovations in this field should continue to lead to novel prognostic and predictive tools.

Functional imaging

MRI and PET can be useful to select patients who are appropriate for early-phase clinical trials that incorporate radiotherapy and biological targeting, or which assess heterogeneity in their morphological and biological responses during treatment. Newly emerging MRI contrast-enhancing agents and PET tracers have the potential to expand the scope of biological imaging that could allow better characterisation of tumour responses to treatment. The value of these contrast agents and tracers as potential biomarkers requires validation in large-scale clinical studies, and linking to the development of specific therapeutic interventions could accelerate this process.

Multiparametric morphological and biological imaging before or during treatment could have greater predictive potential in early-phase clinical trials than any single parameter alone. MRI and PET/CT images are known to contain embedded information that is not visible to human observers but that can be extracted by image analysis algorithms. So-called radiomic approaches can analyse large datasets of standard of care images for features such as heterogeneity, texture, shape, sharpness, compactness, and intensity distribution, which then links these features to prognosis and outcomes.[86] The future potential of radiomics lies not only in the use of these imaging patterns to predict patient outcomes, but also in predicting major driver gene mutations within tumours that could guide specific targeted interventions that can be used in combination with radiotherapy.

Milestones for success in combining targeted therapies with radiotherapy

Combining molecular targeted and immune-modulating agents with radiotherapy continues to show great promise both to radiosensitise tumours and to radioprotect normal tissues. For many promising molecular and immune-modulating agents, their greatest effect in oncology could ultimately rest in their combination with established treatment modalities such as radiotherapy. Our future recommendations to advance the field of radiotherapy–drug treatments are summarised in panel 2.

Panel 2: **Future recommendations on increasing research for radiotherapy and molecular targeted drugs combinations**

Promote a systematic increase in the number and quality of clinical trials that examine combined radiotherapy with molecular and immune targeted drugs using modern clinical design in radiation oncology

Advance the study of precision radiotherapy to achieve individualisation of radiation dose prescriptions on the basis of the genetic and biological features of each tumour and the surrounding normal tissues

Increase dialogue with the pharmaceutical industry to familiarise and prioritise the investigation of new molecular agents in combination with radiotherapy early in the developmental phase of a treatment regimen

Establish clear and relevant clinical endpoints for preclinical and clinical studies of radiotherapy–drug combinations

Develop rigorous in-vitro and in-vivo preclinical models that best reflect radiotherapy-drug treatment approaches in the clinic

Enhance collaborations across radiobiology laboratories to corroborate preclinical models in which radiotherapy–drug combinations can be assessed using similar methodologies and assays to increase reproducibility of preclinical data

Advance systematic focus on radiotherapy quality assurance in preclinical and clinical studies

Establish a learning health system (to include functional imaging and predictive biomarker data) for curative and palliative patients treated with radiotherapy plus molecular targeted agents to assess toxic-effect profiles

Promote research using mathematical biological systems models to optimise radiotherapy–drug treatment schedules on the basis of patient-specific biomarkers

Incorporate imaging and systemic biomarkers in the real-time assessment of tumour response and detection of resistance patterns during therapy

Search strategy and selection criteria

This Policy Review was prepared by a task force nominated by the Leadership of the Science Council of the American Society for Radiation Oncology. The task force contained representatives for the disciplines involved in the diagnosis and care of patients with various forms of cancer. We identified publications through searches of the authors' own files and PubMed for articles published in English after 1970 using search terms including radiotherapy, chemotherapy, combined modality, prognosis, immunotherapy, preclinical models, xenografts, radiotoxicity, and clinical trial methodology. The final list of references included articles, reviews, and books deemed relevant to the broad scope of this guideline.

Conclusion

Ideally, personalised treatment approaches should be tailored for each patient with cancer on the basis of their specific genetic and biological disease features. Oncologists have always strived to treat each patient as unique; however, only in the past decade have molecular and genetic diagnostic tools emerged that can reveal distinct tumour features to guide the design of

high-precision therapies. The advance of molecular targeting drugs and immunotherapies in cancer treatment is taking place in parallel with remarkable advances in the technical precision of radiotherapy delivery. As individualised radiation dose prescriptions based on tumour and normal tissue radiosensitivity emerge, the possibilities of improving the therapeutic ratio of specific agents will further advance. Combining high-precision radiotherapy for local tumour control with molecular targeted drugs and immunotherapies for systemic control provides a powerful opportunity to improve cancer outcomes in the future. Hopefully, radiation, medical, and surgical oncologists working in concert at the forefront of their respective specialties can catalyse this important advance.

Contributors

PMH and RGB outlined the scope and sections and did the final editing of this Policy Review. RGB and HW completed the figures and tables. All authors contributed to the literature search and writing.

Declaration of interests

Before initiation of this paper, all members of the American Society for Radiation Oncology (ASTRO) White Paper Task Group were required to complete disclosure statement, which are maintained at the ASTRO Headquarters in Arlington, VA, USA, and disclosed herein. BA is a consultant for Abbvie. MB is a member of the Scientific Advisory Board for Merck & Co. AD is a consultant for Glenview, a health-care consulting firm. DGK is a consultant for Lumicell Diagnostics and owns stock in Lumicell Diagnostics and XRAD Therapeutics. He has also received research funding from Janssen and Merck for a phase 2 trial. MK receives research funding from Merck. Q-TL owns stock in ALDEA Pharmaceuticals and receives research funding from Amgen. JNS receives research funding from Eli Lily and Genentech. PTT holds patents for compounds and methods of use in ablative radiation therapy. All other authors declare no competing interests. The chairs of this White Paper panel and the Chairs of the Science Council reviewed these disclosures and determined that they did not present a conflict with respect to these panel members' work on this guideline.

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