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Biologically conformal treatment: biomarkers and functional imaging in radiation oncology

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Abstract

‘Conformal radiation therapy’ is the standard of care in radiation oncology, referring to the process of shaping the radiation beam to precisely match a tumor’s physical dimensions. We describe ‘biologically conformal radiotherapy’, in which the radiation oncologist matches the prescribed treatment to a tumor’s biological characteristics and the host’s predicted tolerance of radiation. This paradigm emphasizes that not all tumors are equally sensitive to radiation; conversely, some patients are especially susceptible to radiation’s side effects. Patients bearing radioresistant tumors or those prone to toxicity may be best treated with the incorporation of targeted radiation modulators or, in extreme cases, by a different modality. The biological characteristics of tumors can be assessed by a wide range of techniques: functional imaging (positron emission tomography and advanced magnetic resonance imaging), single gene/protein molecular techniques and ‘omic’ technologies. This paper reviews the latest advances in the use of biomarkers and functional imaging in guiding patients to receive the most appropriate treatment.

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Radiation oncology utilizes ionizing radiation to kill tumor cells and cure cancer. Over the last two decades, there have been two great advances in radiation therapy. The first is the technical ability to focus the beam on the tumor and avoid critical normal structures, by techniques such as intensity-modulated radiation therapy and image-guided radiation therapy. The second is the awareness that radiation combined with chemotherapy, or more recently EGFR antagonists, is more effective than radiation alone. Nonetheless, the therapeutic window is narrow, and radiation when administered in curative doses remains a highly toxic treatment, causing both acute and chronic toxicity.

We should avoid treating patients in whom treatment is likely to be futile or especially damaging. In the pre-biomarker era, gross tumor shrinkage during neoadjuvant chemotherapy was used as a predictive marker of the likely response to radiation. Organ preservation protocols frequently required that tumors that failed to regress undergo radical resection. Another approach was to assess tumor regression midway through radiation treatment (bladder and anal cancer). The use of post-treatment biopsy has also been championed [1]; however, aside from being invasive, the slow mitotic death induced by radiation demands that this be performed long after treatment is concluded, limiting its clinical relevance.

Biomarkers are characteristics that are objectively measured, indicating normal biologic processes, pathogenic processes or pharmacologic responses to a therapeutic intervention. Over the past 5 years, great interest has developed in the use of noninvasive methods to predict tumor and host responses to treatment (Figure 1) [2]. The techniques used range from traditional molecular biology methodology focusing on expression of a specific gene, to genome–transcriptome-wide studies that are dependent on bioinformatics analyses. In this paper we review the literature as it applies to radiation oncology.

Proton emission tomography–computed tomography imaging

Conventional imaging techniques, 2D x-rays and computed tomography (CT), define only anatomical shape and size. The use of intravenous contrast enables an assessment of vascularity and the intactness of the blood–brain barrier. The past decade has witnessed the rapid development of imaging technology. Positron emission tomography (PET) imaging, which today is normally combined with CT, tracks the uptake of a radionucleotide through the body. Scanning patients after the injection of the radiolabeled glucose analog fluorine-18 fluorodeoxyglucose (FDG), one is able to visualize metabolically active tissues. Tumors are typically ‘hotter’ than the surrounding tissue, due to the increased expression of glucose transporters and decreased levels of glucose-6-phosphatase in the malignant cells [3]. Results are presented as qualitative ‘heat maps’. Areas of interest may be semiquantified by use of the standardized uptake value (SUV), a ratio of uptake in the tumor to that in the whole body. Nonetheless, the results may not be as objective as they appear [4]. SUV values vary considerably for individual tumor types, with no clear cut-off between normal and malignant tissue. Aside from tumor biology, blood glucose and insulin levels, as well as the time period between injection and scanning, tumor size and organ movement, all affect SUV values.

Currently, the principal use of PET-CT is to accurately stage patients by detecting distant metastases, both at diagnosis and during follow-up. In the context of radiation therapy PET-CT is increasingly used to delineate local–regional disease, and predict tumor response both during and after treatment, as discussed below.

Advanced MRI

Since its introduction into clinical medicine 30 years ago, MRI has continued to develop. ‘Advanced MRI’ is a collection of technologies that utilize NMR techniques to analyze different physical–chemical characteristics of human tissue. The different techniques are summarized in Table 1. Although potentially applicable to multiple organs, most progress has been made in imaging the CNS.

Biomarker development & validation

Biomarker development is a multiple-step process that begins with a biological hypothesis connecting cause and effect. In order to be useful, a biomarker must be robustly defined, reproducible and shown to consistently correlate with outcome in a range of clinical settings. Validation in an independent patient population is essential. A biomarker will only be valuable if it is found to constructively influence management. Some of these steps have been formally defined by Prentice [5]. Even after the biomarker has entered clinical practice, it may continue to develop, an example is the Oncotype DX[®] expression profiling signature (Genomic Health, CA, USA) used by medical oncologists to predict benefit from adjuvant chemotherapy in breast cancer. Originally defined for node-negative, estrogen-receptor-positive tumors, it was later found to predict prognosis in a far wider spectrum of breast cancers [6]. Biological markers are increasingly used in the clinic by oncologists (Tables 2 & 3).

Few of the biomarkers described in this review have completed the validation process outlined previously, although many are promising candidates. A central problem is cost and logistics. Although the initial steps are fairly cheap and can be completed within a single institution, the later steps of validation and reproducibility require multi-institutional (and frequently multinational) cooperation and substantial financial backing. Biomarkers are increasingly incorporated into prospective Phase II and Phase III studies as secondary end points, enabling large quantities of data to be obtained in an efficient manner (Figure 2).

Predictors of tumor response prior to the initiation of radiation therapy

Hypoxia as a marker of radioresistance

For decades it has been known that hypoxia creates resistance to radiation. More recently, hypoxia has been shown to induce an aggressive cancer phenotype [7]. Studies have correlated hypoxia with poor local control when head and neck [8], prostate [9] and non-small-cell lung cancers [10] are treated with radiation. The standard, but invasive, method of measuring tumor oxygenation is by use of the Eppendorf microelectrode. Contemporary studies have stained biopsy material for the hypoxic marker HIF1- α [9]. In the future, the presence of tumor hypoxia may be an indication for the concomitant use of agents that target hypoxic pathways, a number of which are in development. Hypoxia-sensing radiotracers for use with PET scanners are in clinical trials [11], enabling the localization of intratumor hypoxic islands. These could potentially be ‘dose-painted’ to receive a higher radiation dose [12–14]. Recent studies have emphasized differences between transient and chronic hypoxia [15], with a subset of patients demonstrating shifting areas of hypoxia [12] – complicating the interpretation of single ‘snap-shot’ hypoxia images.

Role of EGFR expression in radioresistance

EGFR is a membrane-spanning protein whose physiological roles include control of cell migration, adhesion and proliferation. In the presence of ligand it becomes autophosphorylated and dimerized, triggering an intracellular cascade that stimulates DNA synthesis and cell replication. EGFR has a secondary function as a regulator of DNA repair, and DNA damage triggers the receptor's translocation into the nucleus. Furthermore, EGFR is activated by radiation, even in the absence of ligand, mediating accelerated repopulation and enhancing DNA repair [16].

EGFR is frequently mutated in human tumors, producing overexpression or overactivity. The level of EGFR expression [17–19] and presence of EGFR polymorphisms [20] have been correlated with radioresistance in a range of tumors including rectal cancer [21,22], glioblastoma [19] and head and neck cancer [23]. Not all studies have demonstrated a correlation, emphasizing the role of other downstream elements, such as PTEN and RAS, in modulating the function of EGFR [24,25]. The relevance of EGFR to radiation oncology is demonstrated by the success of cetuximab, a monoclonal antibody EGFR antagonist, in radiosensitizing head and neck cancer in a Phase III study [26]. In colon cancer treated with cetuximab, the presence of RAS mutation predicts for tumor resistance [27]. Unfortunately, a parallel analysis of the significance of RAS mutation in head and neck cancer patients treated with radiation and concomitant cetuximab has not been performed.

Numerous other molecules have been shown to predict radioresponsiveness, including p21, p53, p63 and p73 (Table 4) [28,29]. In esophageal carcinoma, combined immunohistochemical evaluation of HIF-1 α , p53 and p21 protein expression in the pretreatment biopsy predicted response to combined chemoradiation [30]. Another small study also carried out in esophageal cancer showed that expression levels of the *PERP*, *S100A2* and *SPRR3* genes were predictive of response [31]. These results require validation in larger populations. Khor *et al.* showed that when prostate cancer is treated with radiation, those tumors that over express COX2 benefit from long-term adjuvant androgen deprivation [32].

Blood proteins may also predict response to radiation therapy. A high pretreatment blood prostate-specific antigen level predicts biochemical failure when prostate cancer is treated with definitive radiation therapy, and is an indication for the addition of hormonal therapy or a higher radiation dose. Elevated prostate-specific antigen may reflect the size of the tumor, intrinsic radioresponsiveness or the likelihood that there are distant metastases.

Tumor gene-expression profiling using microarrays

Expression profiling of pretreatment biopsies has been shown to predict complete pathological response to neoadjuvant chemo-radiotherapy in a number of underpowered studies in rectal cancer [33–36]. Likewise in head and neck cancer expression profiling was able to predict locoregional control. Although many of the genes that predict radiosensitivity are concerned with DNA repair, some surprisingly have roles in glucose metabolism and cell differentiation [37]. These studies have typically determined the genetic signature based upon an unbiased analysis of a training set. A major criticism of such studies is their tendency to ‘overfit’ a large number of possible genes to a small number of subjects, weakening the statistical significance of the results. Furthermore, there is little overlap in the candidate gene list produced by different studies. A more successful approach may be to minimize the number of potential covariates by only examining ‘candidate’ genes expected to be important in radiosensitization, and maximize the size of the validation sets. In future, a ‘radioresistant’ signature would be a potential stratification factor in prospective studies, and the patients candidates for more intense therapy/the addition of biological agents.

Role of PET-CT in radiation treatment planning

Conformal radiotherapy, especially intensity-modulated radiation therapy, is characterized by sharp dose gradients. Integrated PET-CT can assist in treatment planning by delineating local–regional disease from normal surrounding tissue. In particular, PET-CT has been found to be useful in distinguishing tumor from atelectasis in lung cancer, and to a lesser extent in assessing mediastinal lymph nodes [38]. Target volumes have been found to increase or decrease in different studies [38,39]. De Ruyscher *et al.* compared target volumes based upon CT or PET-CT imaging; the latter were smaller, enabling the investigators to prescribe a higher dose (69 Gy with PET-CT planning, as opposed to 55 Gy with CT planning) to the target, while keeping within lung, esophageal and spinal cord constraints [39]. There are reports that PET-CT planning decreases inter-observer variability in treatment planning [40,41], although this is contested [42].

A major limitation of PET-CT technology is the inability to image masses smaller than approximately 0.8 cm, hence ‘PET-negative’ structures may nonetheless contain tumor. Daisne and colleagues compared imaging with surgical pathology specimens in pharyngolaryngeal cancer. They found that PET-CT was unable to define superficial extension [43]. A critical unresolved question is: what SUV threshold defines disease? An absolute cutoff of SUV 2.5 or a range of relative cut-offs (40–50%) related to tumor or normal tissue maximum uptake have both been advocated. A related question is how best to integrate the PET-defined target with anatomical contours seen on traditional CT imaging [39,40,44–48]. This problem is keenly felt when there is no sharp margin to the CT component (Figure 3). Ashamalla and colleagues have suggested the use of a ‘halo’ in helping delineate the gross target volume for treatment planning, using a SUV cut-off of 2.5 [49]. Further limitations of current technology relate to organ movement between performance of the CT and PET scan; whereas modern CT scans acquire images within a few seconds, PET images are acquired over a large number of breathing cycles, leading to blurring and inaccuracies in image registration. Unfortunately, no prospective randomized trials have objectively assessed the influence of PET-CT imaging on the safety and efficacy of radiation therapy.

High SUV correlates with poor prognosis in non-small-cell lung cancer [50,51], head and neck cancer [52] and cervical cancer [53]. It is hypothesized that areas of tumor with a higher SUV uptake require a higher radiation dose. Intensity-modulated radiation therapy planning would allow these areas to be ‘painted’ to receive a higher dose [14,54–56]. This approach has yet to be verified in prospective trials. Another complication is that larger tumors often have higher SUV values due to the technical issues of tumor movement and scanner resolution, confounding the interpretation of results.

A large number of labeled molecules for use with PET scanners are in development (Table 5). While the utility of some is in delineating tumor more accurately than FDG, others aim to image aspects of tumor biology such as hypoxia, proliferation and amino acid metabolism. More accurate characterization of tumor biology *in vivo* may influence radiation planning, dose-painting and the optimal scheduling of radiosensitizing drugs with radiation.

Role of advanced MRI technologies in radiation treatment planning

Functional MRI utilizes the magnetic signal variation between oxygenated and deoxygenated blood to indirectly assess brain activity. By performing various tasks during scanning (e.g., memorization, cognitive challenges and watching a movie) researchers are able to map specific functions to different areas of the brain. Functional MRI can be used to map CNS avoidance structures prior to radiation treatment planning [57–61]. Limitations of

the technique include its poor resolution and the limit to the number of tasks that can be visualized in each patient. There is no long-term follow-up data to suggest that functional MRI-based planning minimizes toxicity.

Perfusion MRI and the closely related relative cerebral blood flow quantify blood flow within brain tumors. This can be done using differing techniques such as gadolinium injection or with magnetic pulsation of water molecules. In one study of 23 patients with high-grade glioma treated with radiation, baseline relative cerebral blood flow predicted survival, as did changes within the first 3 weeks of radiation therapy. Those with a low fractional high cerebral blood volume at baseline lived longer [62].

Predictors of tumor response during radiation therapy

An early reading of radioresistance before the conclusion of treatment would be an indication to increase dose, add a concomitant radiation modifier or change treatment modality. Kong *et al.* looked at the role of PET-FDG during radiation treatment of non-small-cell lung cancer in 15 patients. A decrease in SUV during radiation therapy (after 45 Gy) was associated with complete response on post-treatment PET and CT scans [63]. The study was limited by its small size and the late point of tumor assessment – after most of the dose has already been given. Van Baardwijk *et al.* performed PET-CT before, during and after radiotherapy for non-small-cell lung cancer in 23 patients. They found that patients who showed a complete metabolic response in the 2-month post-treatment scan had a much better prognosis than those who showed no response. Interestingly they showed that these two groups behaved differently during treatment. Whereas, the ‘responding patients’ showed a progressive decrease in SUV, the ‘non-responders’ showed an initial increase in SUV, followed by a drop [64]. These two studies are small, but they do signal that one is able to predict long-term response from dynamic changes in SUV during radiation treatment.

Predictors of tumor response post-radiation therapy

Following the conclusion of radiotherapy, there is a rapid fall-off in FDG-SUV values (Figure 4) [65]. A number of investigators have examined the role of PET performed post-radiation treatment in predicting long-term outcome (Table 6). Whereas, some investigators compare pre- and post-treatment SUV values, others simply look at the post treatment scans. While a negative PET scan invariably indicates a good response, a positive PET scan is not always indicative of residual disease. Patients with advanced head and neck cancer treated with definitive radiation frequently undergo a post-treatment neck dissection. A negative post-treatment neck scan is increasingly used as an indication that the operation can be safely avoided [66]. Impressive long-term results have been achieved by Mac Manus *et al.*, demonstrating that complete metabolic response 2 months following treatment for non-small-cell lung cancer correlated with freedom from local failure, distant metastases and death [67]. Disappointingly, he also reported that almost half of those achieving a complete metabolic response eventually suffered local recurrence.

Mardor *et al.* performed diffusion-weighted MRI one-week post-therapy, and were able to differentiate responding from nonresponding malignant brain lesions [68].

Predictors of toxicity to radiation

The likelihood of an individual patient experiencing excessive toxicity from radiation treatment depends on multiple factors, including the volume of normal tissue irradiated, absorbed dose, use of concomitant cytotoxic therapies, comorbidities, gender, patient biological age and the individual’s genetic makeup. Biomarker studies are hampered by the difficulty in quantifying toxicity. Different biomarkers will no doubt be needed in different

tissues for different time points; rectal bleeding and chronic diarrhea are both long-term large bowel toxicities of prostate external beam radiotherapy, yet they represent quite different biological processes. A number of small studies have been performed looking for genetic markers of radiation toxicity as reviewed in [69] (Table 7). Although a number of these studies have shown an association between genetic polymorphisms and toxicity, the small size of the studies and lack of large-scale validation limits the significance of their findings.

Another approach to the prediction of radiation toxicity is the measurement of blood proteins during treatment (cytokines or debris from tissue destruction). High levels would be a potential indication to modify or stop treatment, or alternatively provide preventative measures (e.g., steroids). Blood biomarkers that have been used to predict radiation pneumonitis are circulating antigen KL-6 (a sialylated carbohydrate epitope expressed on type 2 pneumocytes and bronchiole epithelial cells) [70–72], pulmonary surfactant proteins A and D [73,74] and IL-6 [75]. Citrulline (an end product of small bowel metabolism) has been used as a marker of radiation toxicity to the small bowel [76].

TGF- β 1, a peptide with multiple physiological roles, has been shown to be a key inducer of radiation-induced fibrosis. Both the peptide and its receptor are synthesized by almost every cell in the body [77]. Ionizing radiation induces TGF- β 1 production [78,79]. In animals, over-expression of TGF- β 1 results in fibrosis of the kidney, liver and lung. Two alleles within the *TGF- β 1* gene have been correlated with poor cosmetic appearance following postmastectomy radiation [80–82], and may be related to increased rates of secretion [83]. TGF- β 1 tissue levels are elevated for a number of weeks following bowel irradiation, suggesting that it plays a role in chronic radiation enteropathy [84].

In patients receiving lung irradiation, elevated plasma TGF- β 1 levels have been shown to correlate with subsequent lung injury [85,86]. Furthermore, in a multivariate analysis of symptomatic lung injury, elevated plasma TGF- β 1 levels were found to be independent of the lung volume irradiated [87], although the strength of the association has been questioned [88]. The key role played by TGF- β 1 is underlined by the fact that when its signaling is inhibited by molecular knockout [89], an anti-TGF- β 1 receptor inhibitor [90] or by inhibition of integrin $\alpha(v)\beta 6$ [91] (which activates TGF- β 1), radiation-induced lung fibrosis is prevented.

Ex vivo approaches have also been used, in which healthy host cells (typically stimulated peripheral lymphocytes or fibroblasts) are grown and tested for radiosensitivity, transcriptional responses or DNA repair capacity out of the body and the results correlated with patient-experienced toxicity. Studies performed in breast, prostate and nasopharyngeal cancer have led to mechanistic insights: genes belonging to the DNA repair, ubiquitin, apoptosis and stress signaling networks all contribute to radiosensitivity. Nonetheless, this labor-intensive approach is unlikely to enter clinical practice [92–100].

From prognostication to recommendation: a word of caution

The ability to accurately predict who will or will not most benefit from a particular modality is distinct from the ability to predict which modality would be most beneficial. For instance, on discovering that hypoxia is a poor prognostic factor for prostate cancer patients treated with radiation, one could suggest that hypoxic tumors would be best treated by surgical resection. However, surprisingly, hypoxic markers also predict poor prognosis in patients undergoing operative resection [9]. Likewise, hypoxic markers predict a poor prognosis in head and neck cancer whether treated surgically [31] or with radiation [8]. Ideally, a

prospective Phase III trial would be performed to assess the utility of a biomarker-based strategy in selecting treatment modality.

Conclusion

Over the past 15 years, 'conformal treatment', in which the dose distribution of radiation is made to conform to the spatial dimensions of the tumor, has become the standard of care, with a resulting increase in tumor control and decreased toxicity. With the development of effective bio-markers we are witnessing the ushering in of the era of 'biologically conformal treatment' in which radiation, in combination with systemic agents, is made to conform to both tumor and host biological characteristics.

'Biological conformal therapy' requires the active participation of the physician before, during and after treatment completion. The radio-oncologist of the future will require a working knowledge of cancer biology and need to work in close cooperation with medical oncologist colleagues as we move from the era of the 'class solution' to that of 'individualized medicine'.

Future perspective

The next 5 years will see the entry of a large number of biological agents into clinical practice, many of which have been already been shown in the laboratory to combine synergistically with radiation. Molecular analysis of pretreatment biopsies will increasingly be used to guide the selection of the most appropriate agent.

In the world of radiation oncology we will see the increasing use of PET-FDG to define targets for treatment; dedicated PET simulators are already in clinical use. New radiotracers for use with PET scans, enabling the visualization of hypoxia, cell proliferation, cell surface receptors and gene expression, will enter the clinic [101]. Hypoxic areas, as a marker of radioresistance, will be targeted to receive a higher radiation dose using the technique of 'dose painting'.

A competing technology is MRI. Perfusion MRI is able to both accurately classify tumors in a noninvasive manner and predict early radiation failure [62]. MRI spectroscopy is able to image tumor metabolism and has already been shown to be useful in identifying local recurrences amongst patients with prostate cancer [102]. As with PET-FDG, the available technology is far ahead of current practice. Clinical development of new technology requires time, patience, large clinical trials and finance. It is not clear which new technologies will enter everyday practice.

Personalized medicine will become more reliable and more widespread with the adoption of genomic tools such as single nucleotide polymorphism analysis of the host and gene-expression profiling of tumors. Nonetheless, very few biomarkers are entering clinical practice [2]. Ideally the accuracy of biomarker-based recommendations would be tested in Phase III trials; unfortunately, this need is likely to be left mostly unmet.

The challenge for the radiation oncology community over the next decade will be to design and perform the key trials in a timely and rational manner. Thankfully, sophisticated biomarker and imaging modalities are increasingly being integrated into clinical trials [103], bringing the bench and bedside closer together. Many of the studies reviewed in this paper are disappointingly grossly underpowered, and their biomarkers unvalidated. The large studies needed to verify genomic signatures can only be performed in a multi-institutional setting. Furthermore, the progress described here comes at a price: biological agents, radioactive tracers and genomic techniques are likely to remain expensive. In addition,

biological conformal treatment requires more physician input (Figure 5), increasing the workload of an already over-burdened specialty.

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Executive summary

Introduction: individualization of radiation oncology

- Radiation oncology is undergoing a transformation from treating tumors with a 'class solution' to treatment based upon the biological characteristics of the host and tumor.
- This individualized treatment depends upon the development of biomarkers that accurately reflect both tumor and host biology.

Biomarkers

- Biomarkers may be measured before, during or after therapy.
- Their usefulness is dependent on validation with clinical end points in multiple clinical contexts. Very few have entered clinical use.

Evolving biomarkers

- Evolving biomarkers for use in radiation oncology include:
 - Single nucleotide polymorphism analysis of the host genome.
 - Circulating blood proteins.
 - Immunohistochemistry of tumor biopsy.
 - Expression profiling of tumor samples.
 - Hypoxia, as assessed by microelectrode, tissue staining or proton emission tomography (PET).
 - *Ex vivo* approaches, in which tumor biopsy tissue is grown and analyzed in the laboratory.

Functional imaging

- Functional imaging is based upon PET or magnetic resonance imaging technology to accurately delineate tumor from normal tissue, and hence has a role in radiation treatment planning. Furthermore, it is a sensitive indicator of response.
- Functional imaging is increasingly able to demonstrate a tumor's biological characteristics and the quality of the microenvironment in a noninvasive manner.

Predictors of tumor response prior to the initiation of radiation therapy

- Hypoxia is a marker of radioresistance, and can be measured via immunohistochemical staining of biopsy specimens for HIF1- α or by means of specific radiotracers for use with PET scanners.
- Multiple molecules have been associated with radioresistance, the most prominent of which are the EGFR and mutated p53.
- PET-computed tomography imaging is increasingly being used to define the anatomical contours of tumor. In the future, it may be used to locate radioresistant areas within tumors requiring additional treatment.

Predictors of tumor response during radiation therapy

- Changes in the standardized uptake value (SUV; as measured with a PET scan) early in the course of radiation treatment may predict eventual response to treatment.

Predictors of tumor response post-radiation therapy

- A low SUV reading after the completion of radiation therapy is an increasingly used metric of response; however, methods have yet to be standardized and validated.

Predictors of toxicity to radiation

- TGF- β 1 is a key protein responsible for radiation-induced fibrosis in multiple organs.
- In patients receiving lung irradiation, elevated plasma TGF- β 1 levels have been shown to correlate with subsequent lung injury.

Future perspective

- A large number of biologically targeted agents are in development, many of which have been successfully combined with radiation in animal models.
- Sophisticated biomarker and imaging modalities are increasingly being integrated into clinical trials, and may become a condition in a medication's 'indicated use'.

Conclusion

- Radiation oncology is becoming increasingly individualized.
- Disparate technologies are being used to characterize or predict a tumor/host response to radiation in a noninvasive manner.

Figure 1. Biomarkers can be used in multiple aspects of radiation oncology

'Prognostic' refers to the underlying aggressiveness of the cancer; 'predictive' refers to the responsiveness of the cancer to a particular treatment.

Figure 2. Incorporation of biomarkers into prospective trials

Multiple biomarkers are increasingly being incorporated into prospective trials as secondary end points, facilitating the acquisition of good long-term follow-up data. It is also an efficient use of resources. Modified from a diagram created by by Mark A Watson, Washington University School of Medicine, USA.

Figure 3. The role of proton emission tomography-computed tomography images in treatment planning

A 53-year-old male presented with dyspnoea. CT imaging demonstrated a left hilar mass, near-total obstruction of the left lower lobe bronchus and subsequent left lower lobe collapse. The PET-CT image allowed the radiation oncologist to differentiate the tumor from the healthy lung. Nonetheless, the line of demarcation is not as clear as it appears, being highly dependent on the windowing applied to the fluorine-18 fluorodeoxyglucose image (see text: *Role of PET-CT in radiation treatment planning*).

CT: Computed tomography; PET: Proton-emission tomography.

Figure 4. Rapid fall-off in maximal tumor standardized uptake value following completion of radiation treatment to non-small-cell lung cancer

Patients received 42–66 Gy to the lung/mediastinum. Fluorine-18 fluorodeoxyglucose-PET CT scans were performed approximately every 3 months. Each line represents fluorine-18 fluorodeoxyglucose uptake in one particular anatomical site of disease over time [65].

CT: Computed tomography; PET: Proton emission tomography; SUV: Standardized uptake value.

Figure 5. Evolving paradigms in radiation treatment

The current paradigm is based upon the concept of ‘class solution’ for cancers of a particular stage and site. The evolving paradigm of ‘biological conformal treatment’ emphasizes the heterogeneous nature of both tumors and hosts in their response to both radiation and systemic agents. The paradigm demands a high level of physician input at all stages of radiation treatment and follow-up.

Table 1

Advanced MRI technology as applied to radiation oncology.

Modality	Measured quantity	Biological characteristic	Significance to radiation oncology	Ref.
Diffusion-weighted imaging	b value, apparent diffusion coefficient	Free water movement reflecting vasogenic edema and tumor cellularity	Prognostic factor for malignant astrocytoma; early detection of response to radiation; differentiation of radionecrosis from recurrent glioma	[68,105,106]
Diffusion tensor imaging (including tractography)	Fractional anisotropy	White matter tract integrity allowing differentiation of white matter compression from infiltration	Assessment of radiation-induced white matter injury, acute and chronic	[107,108]
Perfusion MR imaging	Relative cerebral blood volume, relative cerebral blood flow	Tumor vascularity	Differentiate the phases of radiation pneumonitis; quantification of tissue perfusion prior to and after anti-angiogenic treatment	[109–111]
MR spectroscopy	Ratio between different chemical peaks	Cell density, proliferation and death	Differentiation of radionecrosis from recurrent glioma; tumor delineation in new and recurrent prostate cancer	[105,112,113]
Functional MRI	Blood-oxygen level dependent contrast	Localization of motor and language function	Critical structure delineation in CNS	[57]
Lymphotropic superparamagnetic nanoparticles	Visual imaging	Lymph node metastatic involvement	Determine gross target volume	[114]

MR: Magnetic resonance; MRI: Magnetic resonance imaging.

Table 2

Biomarkers in clinical use in oncology today.

Biomarkers category	Comments and examples
Blood protein	Prostate-specific antigen produced by prostatic tissue is a prognostic factor in prostate cancer
Circulating tumor cells	Predict recurrence in early breast cancer
Immunohistochemistry of tumor sample	Estrogen receptor in breast cancer predicts response to hormonal manipulation
Fluorescence <i>in situ</i> hybridization analysis of tumor sample	<i>Her2</i> gene amplification in breast cancer predicts response to trastuzumab
Expression profiling of tumor tissue	Oncotype DX [®] is used to determine use of adjuvant chemotherapy in early breast cancer
PCR of tumor sample	Detection of <i>RAS</i> mutation, <i>MGMT</i> methylation
Functional imaging – proton emission tomography	Fluorine-18 fluorodeoxyglucose, a glucose analog that visualizes tumor hypermetabolism, for staging and response to treatment

Table 3

Anticancer agents whose efficacy is governed by biomarker expression.

Drug	Cancer site	Biomarker	Ref.
Hormonal therapy	Breast	Estrogen and progesterone receptor	
Trastuzumab	Breast	HER2/neu	
Adriamycin	Breast	HER2/neu	[115]
Paclitaxel	Breast	HER2/neu	[116]
Erlotinib	Non-small-cell lung cancer	EGFR mutation	[117]
Cetuximab	Colon	PIK3CA mutation/PTEN expression/Ras expression	[118]
Imatinib	Gastrointestinal stromal tumors	c-kit expression	
Temozolomide	Glioblastoma	MGMT methylation	[119]

Table 4

Genes that determine radiosensitivity.

Gene	Function	Ref.
Ribose 5 phosphate isomerase A	Part of pentose phosphate pathway	[37]
Retinoblastoma-binding protein 4	Chromatin metabolism, regulates Ras signaling and cytoskeletal organization	[37]
G-protein signaling regulator 19	May regulate Wnt signaling	[37]
Affymetrix ID HG4236-HT4506	Unidentified	[37]
TATA-box binding protein-associated factor 5	Subcomponent of transcription Factor II D	[37]
DNA topoisomerase I	Nicks, rotates and relegates one of the strands of the DNA double helix	[37]
<i>p21</i>	The p53-dependent cell cycle G1 phase arrest	[30]
<i>p53</i>	Transcription factor and tumor repressor gene known as 'the guardian of the genome', plays a central role in coordinating response to DNA damage	[28,30]
<i>p63</i>	Member of <i>p53</i> gene family	[28]
<i>p73</i>	Member of <i>p53</i> gene family	[28]
<i>HIF-1α</i>	Hypoxia-inducible transcription factor	[30]
<i>PERP</i>	Apoptosis-associated target of <i>p53</i> and <i>p63</i>	[31]
<i>S100A2</i>	Regulator of cell-cycle progression and differentiation, regulated itself by p53 and p63	[31]
<i>SPRR3</i>	Differentiation marker of squamous epithelium	[31]

The list is neither exclusive nor validated. The importance of any individual gene is probably highly context-dependent [104].

Table 5

Current and future radiotracers for use with positron emission tomography.

Functional aspect	Radiotracer	Implications for radiation treatment
Glucose metabolism	Fluorine-18 fluorodeoxyglucose (glucose analogue)	Accurate localization of tumor Assessment of viability post treatment Assessment of normal tissue damage
Hypoxia	[¹⁸ F]fluoromisonidazole, Cu(II)-diacetyl-bis(N(4)-methylthiosemicarbazone	Marker of radioresistance
Amino acid uptake	C11-Methionine, F18-tyrosine, amino acid analogues	Tumor delineation
Tumor-cell proliferation	F18-fluorothymidine	Assessment of viability post-treatment
Apoptosis	^{99m} Tc-annexin	Assessment of viability post-treatment (especially lymphoma)
Drug distribution and metabolism	Labeled drug	Biodistribution in normal and tumor tissue

Table 6

The use of proton emission tomography–fluorine-18 fluorodeoxyglucose imaging to assess response following completion of radiation treatment and correlations with clinical end points.

Primary cancer	n	Tx modality	SUV measure found most useful	When to perform PET	Clinical end point	Results	Ref.
Rectum	44	Neo-adjuvant chemo-RT prior to resection	Comparison of pre- & post-tx SUV	Before tx & 5–6 weeks post completion	Pathological response	PPV 77%, NPV 89%, accuracy 80%	[120]
Nasopharynx	39	Concurrent chemo-RT	Absolute SUV 3 months post tx (cut-off 4.0)	Before tx and at 3 months post completion	LC	Absolute SUV at 3 months after completion predicted LC	[121]
Head & neck	28	Concurrent chemo-RT	Qualitative	Twice: 4–8 weeks and >8 weeks post completion	LC	Sensitivity 76.9%, specificity 93.3%. Second scan more prognostic	[122]
Head & neck	53	RT ± chemotherapy	Qualitative	Within 6 months after completion	Pathological response/LC*	NPV 100%, PPV 43%	[123]
Head & neck	85	RT ± chemotherapy	Qualitative	Various	Pathological response/LC*	NPV 100%	[124]
Mouth	35	Neoadjuvant RT prior to resection	Absolute SUV post-tx (cut-off 4.0)	Not stated	Survival and LC	3-year survival: 80 vs 43%	[125]
Non-small-cell lung cancer	23	RT ± neoadjuvant chemotherapy	Comparison of pre- & post-tx SUV	10 weeks post tx	Survival	1-year survival: 100 vs 20%	[64]
Non-small-cell lung cancer	88	RT ± chemotherapy	CMR	70 days post treatment	LC, distant failure and survival	2-year survival: 62 vs 30%	[67]
Cervix	92	External RT, brachytherapy & concurrent chemotherapy	Qualitative	3 months post tx	Progression-free survival	3-year PFS: CMR 78%, PR 33%, progressive disease 0%	[126]

* End point dependent on whether neck dissection performed.

CMR: Complete metabolic response; LC: Local control; NPV: Negative predictive value; PPV: Positive predictive value; PR: Partial response; RT: Radiation therapy; SUV: Standardized uptake value; tx: Treatment.

Table 7

Candidate genes found to predict for excessive host radiation toxicity.

Gene	Function
<i>GSTP1</i>	Role in detoxification by conjugating compounds with glutathione. Also involved in cancer development
<i>TGFB1</i>	Autocrine growth factor involved in control of proliferation, differentiation, apoptosis and fibrosis
<i>SOD2</i>	Part of antioxidant defense mechanism
<i>XRCC1</i>	DNA repair of single strand breaks
<i>XRCC3</i>	DNA repair protein involved in homologous recombination
<i>XRCC5</i>	DNA repair protein involved in nonhomologous end joining
<i>ATM</i>	Coordinates response to DNA double-strand breaks
<i>APEX</i>	Involved in the DNA base excision repair pathway
<i>ERCC2</i>	Part of nucleotide excision repair pathway
<i>ERCC4</i>	Part of nucleotide excision repair pathway
<i>DNA ligase4</i>	Complexes with <i>XRCC4</i> , catalyzes the final step in the nonhomologous end-joining DNA double-strand break repair
<i>CYP2D6</i>	Xenobiotic-metabolizing enzyme

Data taken from [69].