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Critical Review

Proceedings of the National Cancer Institute Workshop on Charged Particle Radiobiology

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In April 2016, the National Cancer Institute hosted a multidisciplinary workshop to discuss the current knowledge of the radiobiological aspects of charged particles used in cancer therapy to identify gaps in that knowledge that might hinder the effective clinical use of charged particles and to propose research that could help fill those gaps. The workshop was organized into 10 topics ranging from biophysical models to clinical trials and included treatment optimization, relative biological effectiveness of tumors and normal tissues, hypofractionation with particles, combination with immunotherapy, "omics," hypoxia, and particle-induced second malignancies. Given that the most commonly used charged particle in the clinic currently is protons, much of the discussion revolved around evaluating the state of knowledge and current practice of using a relative biological effectiveness of 1.1 for protons. Discussion also included the potential advantages of heavier ions, notably carbon ions, because of their increased biological effectiveness, especially for tumors frequently considered to be radiation resistant, increased effectiveness in hypoxic cells, and potential for differentially altering immune responses. The participants identified a large number of research areas in which information is needed to inform the most effective use of charged particles in the future in clinical radiation therapy. This unique form of radiation therapy holds great promise for improving cancer treatment.

Introduction

Particle (ion) therapy, delivered with protons or heavier ions such as carbon, has distinct physical advantages. ("Particle," "charged particle," and "ion" have been used interchangeably in the present report.) It is also apparent that radiation therapy (RT) with protons and especially heavier charged particles has additional therapeutic potential owing to their biological and immunogenic properties. To further explore these potential advantages, the National Cancer Institute (NCI) hosted a workshop on charged particles on April 5 and 6, 2016. The participants included radiation oncologists, radiobiologists, and physicists (listed in the Acknowledgments section). Among them were representatives of the NCI, Department of Energy, and the White House Office of Science and Technology Policy.

The rationale for the workshop was to discuss our current knowledge of the radiobiological aspects of particles used in cancer therapy and to identify gaps in our knowledge that could hinder particle therapy's clinical effectiveness. Attendees agreed that major gaps exist in our knowledge of the biological characteristics of ions. The relative biological effectiveness (RBE; relative to photons) of ions is a complex function of multiple variables, including not only numerous biological, but also physical variables. It is essential that this functional dependence be quantified and considered in the selection of patients for treatment, during the treatment planning process, and in the analysis of clinical outcomes after particle therapy. Such research will allow the field to maximize the effectiveness of ion treatments. The need for improved understanding of particle RBE (and, therefore, the need for this workshop) has been amplified by the introduction of intensity modulated particle therapy (IMPT). IMPT is a powerful radiotherapeutic technique but each of its beams has a highly heterogeneous dose distribution, which magnifies the effects and complexities of the RBE concept. In contrast, the inherent flexibility of IMPT offers the opportunity to capitalize on RBE through the incorporation of such information into the IMPT plan optimization process, presumably leading to more effective treatment.

Clinically, the most commonly used charged particle is the proton. In the current practice of proton therapy, the RBE of protons relative to photons is simplistically assumed to have a spatially invariant, generic, constant value of 1.1 for all situations. This assumption is based on the averaged data from a number of historic experiments performed under limited conditions (1, 2). This value is used when computing radiation dose distributions for planning proton treatments and for making treatment decisions. Increasingly, it has been recognized that RBE can vary substantially along the path of a proton beam. Thus, the biologically effective dose distributions actually delivered can be significantly different from those planned, which can lead to suboptimal treatments and unforeseen local failures or toxicities. Although the RBE of heavier ions is considered to be variable, the accuracy of the data and the reliability of the RBE predictive models required for clinical applications are inadequate and can lead to problems similar to those for protons.

The objectives of the workshop were to

- Review ongoing or completed clinical trials of particle therapy
- Review the current knowledge of particle biology and identify gaps in knowledge
- Identify limitations of current tools to incorporate biological knowledge into particle therapy
- Review the clinical consequences of such gaps and limitations
- Define future research to study the biological effects of particles to make particle therapy maximally effective
- Make recommendations to the NCI regarding developing research programs focused on biological aspects of particles

The workshop was organized into 10 topics, with each assigned a 1-hour slot and led by 2 comoderators (see the Acknowledgments section). The following sections summarize the discussions.

Clinical Trials

Numerous clinical trials of particle therapy, completed and ongoing, have been performed. Clinical trials of proton therapy, the most commonly used particle, dominate the current picture. Given the lack of heavy ion centers in the United States, all but 1 ongoing clinical trial in the United States involved proton therapy. However, investigators in Asia and Europe have been making significant strides in the study of clinical outcomes after heavy ion therapy. For both proton and heavy ion therapy, the vast majority of trials have been early phase, noncomparative trials. This, along with additional factors such as continued technological advancement, have made it difficult to define the role particle therapy should play in the treatment of cancer. Discussions at the workshop focused on completed or ongoing phase II or III trials comparing particle and photon therapies and the significant obstacles that make the conduct of such trials difficult.

Proton therapy, which is increasingly available, is used for numerous common malignancies such as prostate cancer, breast cancer, lung cancer, and others. Against significant odds, single-institutional studies and large comparative trials have been initiated or, in select circumstances, completed in such diseases (3-17). These include randomized phase II studies of protons versus intensity modulated RT (IMRT) for non-small cell lung cancer (NSCLC) and glioblastoma, the results of which have not yet been reported (18, 19). Larger studies, including a randomized phase III study of NSCLC and a large phase II trial in glioblastoma, are currently underway under the auspices of NRG Oncology. Other newly initiated trials include large randomized phase III trials of proton versus photon therapy for breast cancer assessing differential cardiac toxicity and for prostate cancer assessing differential bowel toxicity. Other comparative studies currently advancing through both institutional trials and cooperative groups include trials comparing the benefits of protons for patients with oropharyngeal cancer, hepatocellular carcinoma, lower grade glioma, and esophageal cancer assessing both normal tissue toxicity and disease control rates.

Although numerous centers and patients now have access to proton therapy, it is important to note that this was not the case until recently. This has inherently limited the conduct of clinical trials. As more proton centers come online, the number of patients enrolled in high-quality trials will hopefully increase. In the United States, insurance coverage for proton therapy can be difficult to obtain. If patients enrolled in prospective trials are not provided coverage, sufficient, high-quality data necessary to define the benefits of proton therapy will never be obtained.

For carbon ions, which have significantly greater biological effectiveness compared with photons or protons, substantial interest exists in the treatment of tumors historically deemed radiation resistant. Because no heavy ion centers exist in the United States, the conduct of trials by US investigators is exceedingly difficult. However, investigators in Europe and Asia have made significant advances (20-25), including numerous early phase clinical trials, most in Japan, with results suggesting increased disease control rates and improved survival outcomes compared with photons. In Germany, investigators from the University of Heidelberg and German Cancer Research Center have numerous ongoing comparative trials. In most of such trials, carbon ions are typically used as a boost after photon therapy. Although clinical outcomes are not yet available, mixed modality treatments could obscure the potential clinical benefits of heavy ions. One notable trial, which is being conducted with the support of the NCI, will address the potential benefits of carbon therapy for pancreatic cancer. This represents a substantial effort by US investigators and their counterparts in Shanghai, where the clinical treatments will be administered. The initial early-stage clinical results of carbon ion therapy have suggested improved outcomes, perhaps based on the unique biological and immunogenic aspects of heavy ions. However, only high-quality comparative trials will be able to produce convincing data to support the continued use of such a costly therapy.

The discussion highlighted the difficulties of conducting high-quality clinical trials for heavy or light ions. One particular topic focused on the rapidly changing technologies for the delivery of particle therapy (eg, IMPT). All completed and most ongoing clinical trials comparing proton therapy to photons have used passive scattering technology. Any proton therapy modality, compared with photons, will be associated with reduced low-dose exposure to normal tissues. During the preceding decades, it was continually argued that this will translate into a reduced incidence of secondary malignancies or reduce organ dysfunction. However, it is important to highlight that, with passive scattering, high-dose conformality can be significantly worse than IMPT or IMRT. Although low-dose sparing is improved with either proton therapy technique, if normal tissues in close proximity to target volumes receive higher doses of radiation with passive scattering, some toxicities could be increased. Additional issues discussed included difficulties with funding and lack of insurance coverage, as described. The final and perhaps most important topic of discussion centered on how to relate differences in the biological effects of protons versus photons to clinical outcomes. As indicated in the Introduction and described in subsequent sections, our knowledge of proton biology is surprisingly sparse and based nearly entirely on animal or in vitro studies. Clinical evidence of differential biological effects is needed. Currently, nearly all trials have focused on purely clinical outcomes such as disease control, survival, or a reduction in adverse events. However, if sufficient appropriate treatment response data are collected and analyzed methodically, such trials could also provide clinical evidence of systematic deviation of RBE from 1.1 and variations in both tumor and normal tissue responses between

particle therapy and photon modalities. Examples include assessing the positron emission tomography (PET) response within primary lung tumors and in normal lung exposed to higher linear energy transfer (LET) regions in the distal end of proton beams. For brain tumor patients, post-treatment magnetic resonance imaging can also reveal the tumor response, as well as subclinical normal tissue damage. For all such studies, high-quality, reproducible, serial imaging studies during and after proton therapy is important. The involvement of radiation physicists is essential to ensure the accuracy of doses delivered and to generate LET distributions required to model the biological effects.

Throughout the history of radiation oncology, a series of technological advances have incrementally improved our ability to conform radiation doses to discrete target volumes. Even within photon therapy, remarkably little prospective evidence exists to support the use of advanced modalities such as IMRT. Instead, their use has been based on first principles, namely, high doses to targets and low doses to normal tissues. In contrast, evidence for particle therapy has been increasingly demanded by radiation oncologists, other physicians, insurance providers, and the health care field in general. Although the conduct of high-quality clinical trials to provide such evidence is challenging, it is important to pursue such endeavors to define the role of particle therapy and advance our field as a whole.

Biophysical Models and Open Questions

As noted in other sections, our knowledge of the RBE of both heavy ions and protons is increasing. Biophysical models of the RBE relative to a low LET reference radiation (megavoltage x-rays or ^{60}Co γ-rays) could enhance our ability to individualize and more fully exploit the potential of particle therapy. This session focused mainly on proton therapy, for which, currently, a generic value of 1.1 is used clinically.

For fraction sizes that are small compared with α/β, the accumulated evidence from empirical studies (2, 26) and mechanistic considerations (27, 28) suggests that proton RBE increases in an approximately linear fashion as a function of LET up to the tip of the Bragg peak (in the region from ∼2 to ∼15 keV/μm) and beyond for endpoints such as DNA double strand break induction (27, 28) and clonogenic survival (27-30). Beyond the tip of the Bragg peak, and for light ions, the RBE for double strand break induction and clonogenic cell survival increases in a monotonic, nonlinear and particle-specific way up to at least ∼100 keV/μm and then reaches a plateau or shows a trend downward (27-31). Particle biological effectiveness relative to a low LET reference radiation (same oxygen concentration) tends to increase in a monotonic, nonlinear, and particle-specific fashion with decreasing oxygen concentrations (27, 28, 31, 32).

Uncertainties in RBE estimates from in vitro and in vivo studies are substantial in part because of nonstandardized reporting of reference and particle beam dosimetry (33, 34). Additional preclinical (in vitro and in vivo) studies are needed to examine particle RBE in the limit of a small dose per fraction (<1-2 Gy) and for doses that are large compared with α/β. The latter studies are needed to differentiate among alternate models and mechanisms of action and to aid in the clinical implementation of hypofractionated particle therapy (35, 36). Preclinical and retrospective clinical studies are also needed to examine whether trends in particle LET or the RBE for key molecular and cellular endpoints suffice for the biological optimization of clinical endpoints, especially in view of variations in tumor and normal tissue radiosensitivity among patients. Preclinical studies of the effects on particle RBE of targeted chemical and immunologic treatments combined with radiation are also needed. Standardized absolute dosimetry, microdosimetry, and Monte Carlo track structure simulations, including the effects of secondary ions, are essential to aid in the interpretation and effective analysis of data from laboratory and clinical studies.

Preclinical and clinical studies are needed to examine and separate dose−volume effects from the effects of particle RBE to develop and validate equivalent tolerance dose constraints for conventional and hypofractionated particle therapies. Clinical studies are needed to examine whether spatial variations in particle RBE within organs at risk correlate with unexpected treatment effects. Because toxicities are relatively rare events, complimentary animal studies are needed to aid in the interpretation of the RBE for the clinical endpoints. However, even in the absence of additional clinical evidence, LET distributions (37, 38) or molecular or cellular endpoints that are surrogates for clinical outcomes (39-42) could be used to guide the minimization of biological effects in critical structures to exploit the unique biological characteristics of particles and improve the therapeutic ratio using IMPT.

Treatment Response and Optimization

The main focus of the treatment response and optimization session was on the RBE of protons. The rationale for the assumption of RBE of 1.1 and its continued use in the current practice of proton therapy is manifold: (*1*) large uncertainties exist in RBE, in particular with regard to its dependence on individual patients; (*2*) the assumption that higher RBE affects only a small region near the distal edge; and (*3*) no clinical evidence of harm is available to date to necessitate a change. In contrast, it is evident that proton RBE is variable, increasing from ∼1.0 at the entrance into the tissue to ∼1.3 to 1.4 at the Bragg peak of a monoenergetic beam. Substantially greater values on the order of 4 have been reported in the distal falloff region (43). This region is rather steep in water but is degraded in tissue, in particular, when a beam passes through a complex heterogeneity (44), and can spread over a large region, especially in a low-density medium such as lung tissue. Regarding the lack of clinical evidence, one can argue that the "absence of

evidence is not evidence of absence." It is plausible that the evidence is obscured by uncertainties related to anatomy variations, approximations in dose calculations, anatomy delineation, and patient-specific factors. Nevertheless, as the number of patients treated with protons increases, the reports of unforeseen toxicities have been increasing.

To unequivocally demonstrate the advantage of protons, it is essential to understand the effect of the variability of RBE on treatment response. This, in turn, would require that, to the extent possible, other sources of uncertainties are mitigated and residual uncertainties are incorporated into computed dose distributions. The correlation of more accurate estimates of dose distributions actually delivered thus obtained with treatment response indexes might reveal the quantitative information about RBE variability. The resulting improvements in our understanding should lead to the development of more reliable models of predicting RBE as a function of dose, LET, and α and β values. Current models are simplistic and typically result in an RBE that is a linear function of LET (2, 26, 39, 45-48), which is not consistent with the results of recent high precision experiments. An approximation in these models is that they assume the RBE is a function of dose- or track-averaged LET. Consequently, they underpredict the RBE in regions near and beyond the Bragg peak.

Reliable models are essential for evaluating the potential clinical effect of dose distributions produced by a treatment planning system. They are equally important for optimizing the IMPT dose distributions to maximize the biological effect (cell kill) within the tumor target for the same physical dose and to minimize the biological damage outside the target. The main rationale for using protons is the characteristic Bragg curve. Because the RBE-weighted dose at the Bragg peak is 30% to 40% higher than the entrance dose compared with the physical dose, proactive incorporation of a variable RBE into the treatment planning process could lead to an even greater differential between the target and normal tissue doses compared with the assumption of a constant RBE of 1.1. One method to achieve this differential might be to perform IMPT optimization using criteria defined in terms of the RBE-weighted dose computed using a variable RBE model. Even a simplistic model could direct greater RBE protons into the target and away from normal tissues. An alternative strategy would be to base the optimization criteria on dose*LET to minimize LET in critical normal tissues (38, 49). Those who favor the latter over the former approach have argued that uncertainties in RBE are high. They assert that a need exists to provide the physician something that is accurate. Thus, the plan evaluation and optimization should be based on physical parameters such as the dose and LET, which can be calculated with a greater degree of certainty, and we should continue using an RBE of 1.1. Moreover, they maintain that the dose*LET approach is much simpler to use and that using a variable RBE could lead to the adoption of different models at different institutions, leading to inconsistencies in the reported results.

However, one can argue that the use of an RBE of 1.1, which is, in fact, the simplest of the models, has even greater uncertainty than the use of variable RBE. Little doubt exists that RBE increases with depth as the protons slow down. Thus, a relatively simple consensus model could be adopted and might, for instance, have an entrance RBE of 1 and an RBE of 1.2 to 1.4 at the Bragg peak, depending on α and β values, which should, in principle, lead to safer and more effective treatments. During the transition, one could represent the results in terms of RBE 1.1 to ensure the safety of normal tissue doses and acceptable target dose heterogeneity.

The ongoing debate indicates the need for considerable further research to fill large gaps in our knowledge of RBE based on in vitro and in vivo experiments and from correlations of various clinical and imaging response markers with accurate estimates of dose distributions actually delivered. A need also exists to refine existing models, or develop new ones, for predicting RBE of not just clonogenic survival but also other endpoints for tumors and normal tissues. Additional research and development is also necessary to intercompare and determine the best approach to incorporate the biological effect models for the evaluation of computed dose distributions and for optimization of IMPT. Finally, it is necessary to evaluate the effect of such factors as inter- and intrafractional anatomy variations on biological consequences and in regions affected by distal edge degradation. Such research is critical to exploit and demonstrate the true potential of proton therapy.

Although most of the discussion centered on protons, similar issues also apply to heavier ions. It is important to note that variable RBE models and RBE-weighted optimization of IMPT are already in use for carbon therapy; although the RBE data and models are in need of considerable improvement.

Other aspects of biological optimization of particle therapy discussed in this session included the need to better define the clinical target volume (CTV). The steeper dose gradients that are possible with particle therapy make the CTV less forgiving of uncertainties compared with conventional photon therapy. Finally, the greater dose shaping potential of particle therapy can enable new methods to deliver dose over time (ie, different dose fractionation schemes). To exploit this potential fully, the spatial and temporal dose distributions can be optimized simultaneously using biological models such as the linear-quadratic model (50). This spatiotemporal optimization approach appears to be particularly promising when combined with the optimization of chemotherapy or immunotherapy schedules.

RBE of Normal Tissues

Charged particle irradiation can cause significant alterations in critical tissues such as the central nervous system and cardiovascular system at relatively low doses (51-53).

Hence, although charged particle therapy is designed to deliver a high dose to tumors and limit the exposure of normal tissues, microdosimetric differences between radiation modalities can elicit unintended short- and long-term normal tissue complications. Although preclinical studies have suggested the potential for charged particle therapy to precipitate adverse normal tissue effects, the field lacks rigorous comprehensive studies in this area. Although much is known about the responses of normal tissues to photon irradiation, detailed understanding of the critical underlying mechanisms remains unclear, and how the processes might change with changes in LET has not been well elucidated. It is also unclear how previously irradiated tissues might cope with a second insult or injury (54, 55). To examine the biological mechanisms of radiation response, focused studies need to be designed to characterize radiation toxicity in multiple tissues and organ sites after clinically relevant fractionated irradiation paradigms. Hence, a variety of small and large animal model studies is required to bridge the gap from preclinical to clinical research.

Although the concept of RBE was originally developed on the basis of radiation cell survival, RBEs are currently also used to aid in the comparisons of normal tissue responses to various types of ionizing radiation. However, each normal tissue shows a variety of radioresponsive endpoints that do not all depend on radiation-induced cell death (56). Rather, varying underlying mechanisms could be involved, including vascular injury and endothelial dysfunction, structural alterations to neurons, mitochondrial alterations, and chronic inflammation. Currently, no consensus has been reached regarding which endpoints are most relevant for the determination of normal tissue-specific RBEs.

In addition to the biological endpoint, as noted, RBE values also depend on factors such as the dose depth and LET distribution (1, 2). This is particularly relevant for charged particle therapy, because the radiation fields are more complex than in conventional photon irradiation paradigms. Although normal tissue injury and its dependence on LET have been investigated using certain cultured cell systems (57-59), in vivo studies in this area have been more limited, and our knowledge of LET-dependent effects in normal tissues is correspondingly low. Moreover, although the mean dose or simple dose−volume constraints to an organ at risk are commonly used in RT planning, the relevance of such indexes is in question. The dose to substructures within an organ might be more informative toward the normal tissue radiation risk. For example, the structural integrity of critical neural circuits mediating neurotransmission between distinct subregions of the brain could exhibit differential radiosensitivity and LET-dependent effects. This highlights the need for additional research to examine whether spatial variations in RBE within organs at risk can explain adverse treatment outcomes.

Biological Issues in Hypofractionation With Particle Therapy

X-ray stereotactic body RT (SBRT) is moving hypofractionation toward the delivery of 1 to 5 fractions (oligofractionation) with very high doses/fraction ($\leq$25-30 Gy). For NSCLC and oligometastases, SBRT has shown high control rates, durable local control, and few normal tissue complications (60). At a very high dose, the vascular injury (ie, damage to the endothelial cells supplying the cancer tissue with oxygen and nutrients) might become a dominant pathway for tumor suppression (61). Normal tissues can now be spared to a greater degree using modern image-guided techniques, at least for parallel tissues (eg, lung), enabling the safe delivery of hypofractionated treatment.

Hypofractionation requires technology to better localize the high and intermediate dose and improved imaging modalities to reduce the target size (eg, less need for prophylactic coverage). Studies of intermediate-range hypofractionation in common diseases such as lung, breast, colorectal, and prostate cancer have shown benefits and harm similar to those of less-convenient protracted courses (60). A more potent ablative range dose per fraction has even been tolerated in frail patients and has become a standard treatment in several common cancer presentations (62). With equivalent or improved tumor control compared with conventionally fractionated radiation, improved patient satisfaction with fewer trips, and less usage of expensive radiation delivery equipment, hypofractionation has been shown to be considerably more cost-effective and clinically beneficial compared with competing therapies (36, 63, 64).

The greater conformality achievable by the physical characteristics of charged particles makes them ideal for hypofractionated or radiosurgical treatments (65). However, particle radiobiology research at high doses is needed to support and guide oligofractionation in particle therapy. Heavy ions can be an ideal tool for hypofractionation compared with, not only photons, but also protons. Currently, most carbon ion treatments are hypofractionated. Physically for heavy ion therapy, reduced lateral scattering leads to lower doses to normal tissues around the CTV; therefore, a high dose/fraction can be better tolerated. High LET radiation-induced cell killing is poorly dependent on the cell-cycle phase and on the oxygen concentration compared with low LET protons and photons (66). The RBE at a high dose/fraction is reduced compared with low doses; however, the reduction can be more significant for normal tissues, which generally have lower α/β ratios than those of tumors (35). The optimum use of heavy ions in hypofractionation requires careful selection of beam angles; therefore, rotating gantries would likely be beneficial. Hypofractionation also mandates greater emphasis on a reduction of the physical uncertainties associated with particle therapy. In addition, although radiation sensitizers

are less necessary for hypofractionation, given the impressive tumor control, a legitimate radiation protector could be useful, especially if treating tumors next to serial structures (eg, bowels, airways, spinal cord). Further research into the types of radioprotectors necessary for use with heavy ions, compared with photons, is essential because the cellular damage can differ substantially between radiation types.

Unlike the historical experience in the United States and recent experience in Europe, Japanese investigators have migrated toward hypofractionation with heavy ion therapy. This makes perfect sense because feasible hypofractionation requires a conformal high dose and compact falloff of the intermediate dose, features that can be effectively achieved with heavy ion therapy. Although no heavy ion clinics have yet provided high-level clinical evidence, the results from Nuclear Information and Resource Services and other Japanese centers are impressive (21, 67-69).

High-level phase III trials have been notoriously difficult to perform owing to problems with physician or patient lack of equipoise, in particular, if comparing disparate therapies. Thus, rather than compare surgery to carbon ions or photons to carbon ions, it might be more successful to compare, for example, 30 fractions to 15 fractions with the idea that heavy ions would allow shorter treatment courses to be completed with less toxicity. In tumors with predicted poor outcomes because of molecular features or hypoxia, heavy ions could be tested to better exploit the reduced oxygen enhancement ratio (OER) (70). With the better geometric distribution of dose related to heavy ions compared with photons, and even protons, hypofractionated heavy ions could also be considered for adding local therapy in the treatment of metastatic cancer (71). This short-course, hypofractionated approach might be consolidative (eg, after a partial systemic therapy response) (72) or as a conditioning regimen (eg, debulking large tumors or stimulating the immune system) (73, 74) in a broad group of patients with metastatic disease.

Immune Response and Particle Therapy

Cancer RT results in a delicate balance of immune stimulation and immune suppressive effects. RT is an effective inducer of immunogenic cell death (ICD), which is defined as translocation of calreticulin to the tumor cell membrane (75), the release of HMGB1 (76), and the release of adenosine triphosphate by dying tumor cells, which leads to inflammasome activation, secretion of interleukin-1β, and priming of interferon-γ to produce activated $CD8^+$ T cells (77). Radiation-induced ICD is dose dependent and can be detected in the dose ranges used in the clinic (78). For particles, the LET also has an important role.

A multiplicity of radiation effects are sensed by the immune system. Radiation primes dendritic cells, which then activate cytotoxic T cells, which then attack the process-initiating tumor cells (in situ vaccine effect) (79-81). Furthermore, these activated T cells can provide immunity against metastasis or initiate a dormancy of metastatic cells. Immune suppressive effects are also induced by radiation. Many tumor cells have large amounts of inactive transforming growth factor-β (TGF-β) that is sequestered by latency-associated peptide. With irradiation, TGF-β is released, leading to the inhibition of dendritic cells and the inhibition of T-cell effector function. Furthermore, the priming of T cells requires TGF-β blockade (82). Immunosuppressive effects of radiation are also related to the number of circulating blood cells exposed during RT. For example, in patients with high-grade glioma treated with 6 weeks of RT and temozolomide, protracted lymphopenia was common, and reduced survival was associated with a CD4 count of $<200/\mu L$ (83). Yovino et al (84) have introduced a model to predict the radiation dose to circulating T cells. The model is based on the number of radiation fractions, dose rate, and field size (84). They focused on the effect on circulating naive T cells, because they are among the most radiosensitive cells in the body. The overarching hypothesis has been that extended treatment times to larger volumes will substantially reduce the lymphocyte pool from which an immunologic response might be mounted. Similar effects were observed in lung tumors, with larger gross tumor volumes correlating with lower lymphocyte nadirs and overall survival after chemoradiation (85), and in pancreatic cancer, in which hypofractionated stereotactic radiation regimens eliminated the lymphopenia associated with standard-fraction chemoradiation therapy (86, 87). Radiation-induced lymphopenia might also be driven by irradiation of reservoirs of lymphocytes, such as the spleen, heart, and bone marrow (88). Collectively, these data point to tumor-specific, treatment-specific, and tumor location-specific parameters that influence the likelihood of patients developing lymphopenia and, by inference, the inability to mount a systemic immune response.

One question is whether charged particles elicit a different and/or distinct immune response than that observed with photon irradiation. An initial analysis of proton versus cobalt irradiation of a panel of prostate, breast, lung, and chordoma cancer cells noted no appreciable difference in the upregulation of surface molecules involved in immune recognition and ICD (89). No appreciable difference was noted between carbon ion beams and photons for the extracellular release of HMGB1 when controlled for iso-survival doses (90). In the absence of a more comprehensive assessment of immune phenotypic changes after charged particle radiation, this raises the question of LET and its effect on radiation-induced ICD. Preliminary experimentation, as a part of a collaboration between Cornell and Columbia Universities, using protons and deuterons generated using the Radiological Research Accelerator Facility, has identified increased HMGB1 release in tumor cells receiving 5 Gy of protons or deuterons (each with LETs of 35 keV/μm) compared with the same dose of 5 Gy delivered with 50 kV x-rays. Whether an

increase occurred in the release of HMGB1 with deuterons of increasing LET was inconclusive. Further experiments are ongoing at the Radiological Research Accelerator Facility and other facilities. In summary, dose (high), dose rate (high), fraction number (limited), volume of tissue irradiated (low), and LET are all parameters that should be considered when radiation is applied to stimulate the immune system or in concert with immunotherapy.

Another area of interest is combining radiation therapy with immune checkpoint inhibitors. This is based on the recent recognition that tumors with a high synonymous mutation burden are more likely to respond to anti-CTLA1 or anti-PD1 therapy in melanoma and NSCLC, respectively (91, 92). This effect was even more pronounced when the ability to elicit a neoantigen signature was considered. Furthermore, greater responses to immune checkpoint inhibition were noted in patients whose tumors harbored mutations in genes controlling DNA repair, replication, and maintenance of genomic integrity. These findings hint at the tantalizing possibility that clustered DNA damage, such as is commonly observed with charged particle radiation, might result in more unrepaired DNA damage and thereby create a genomic landscape within tumors that parallels that observed with high mutation-burdened tumors. Furthermore, chemotherapy-resistant cancer stem cells might be preferentially sensitive to irradiation with charged particles compared with photons by an increase in the production of reactive oxygen species and irreparable clustered DNA damage (93-95). Whether a qualitative difference in clustered DNA damage between photon and charged particle radiation can be exploited for priming tumors to immunotherapy remains unconfirmed, with recent evidence suggesting that a response to immunotherapy is more likely in tumors harboring clonal neoantigens rather than the subclonal neoantigens often induced by cytotoxic chemotherapy (96).

"Omics" and Biomarkers in the Charged Particle Therapy Space

Omics technology is an integral tool for the development of a knowledge base that should allow for accurate diagnosis of, and personalized treatment selection for, cancer. The promise of integrating omics analysis into radiation oncology includes the development of biosignatures or biomarkers that are predictive or prognostic of the therapy outcome. These include normal tissue risk and the identification of molecular drivers of the therapeutic response to radiation because identifying patients whose tumors are radioresistant to x-ray therapy might make such patients ideal for ^{12}C ion therapy. Also, the integration of omics analysis into radiation oncology includes, ultimately, identifying molecular targets that would suggest drug and radiation combinations or second-line therapies.

The purpose of this section was to highlight the use of "omics" technology (ie, genomics, epigenomics, proteomics, metabolomics, panomics) to describe the response of cells, tissues, and animals to charged particle exposures and to identify some of the challenges that lie ahead in the use of omics to develop biomarkers for the response of tumors and normal tissue to therapeutic exposures of charged particles. Currently, the omics data available in reported studies describing the response of cells or tissues to charged particles at doses or energies applicable to charged particle therapy are limited. Of the available omics data, most have been generated through the National Aeronautics and Space Administration Radiation Health Program, in which the ions and energies are those found only in deep space. These are the so-called HZE ions, which are the high-energy nuclei in cosmic rays and have a charge much greater than 2. Most have no application for therapy (with the exception of ^{12}C and ^{16}O); and the doses used are also lower than those used in RT. Still, these data have identified temporal gene expression patterns that are similar to those seen with x-rays or γ-rays, as well as those unique to the heavy ion used (^{28}Si, ^{56}Fe) (97, 98). When ^{12}C was compared to x-rays, both common and unique gene expression patterns were also seen, with overlap with the gene expression patterns seen with particles of much higher Z and energy, although the expression patterns were limited to single doses and times after irradiation (99-101). The suppression of proangiogenic gene expression after proton exposure, which is not seen after x-ray exposure (102), is now supported by ^{12}C irradiation of cells in culture, where expression analysis has revealed the lack of induction of hypoxia inducible factor (HIF)-1 (103) and stem cell factor expression (104), both of which are associated with angiogenesis. Furthermore, the suppression of migration and invasion has been demonstrated in lung cancer cell lines after carbon ion irradiation (105). The stratification of cellular response to charged particle exposure has also been demonstrated at the epigenomic level, where micro-RNA signatures for radiation type, proton dose, and time after proton exposure were identified in circulating micro-RNA isolated from the blood of irradiated mice (106).

Metabolomics is a promising and relatively recent addition to the omics field that offers opportunities to develop biomarkers for the response to particle therapy and to understand the effects of malignant change on cellular metabolism. In the case of the National Aeronautics and Space Administration-supported research, major findings have been that HZE ions, including carbon, show differing cellular sensitivity than photons and protons (eg, hematopoietic progenitor cells are exquisitely more sensitive to HZE ions) (107). In addition, HZE ions show marked long-term effects on cellular signaling in diverse tissues, including gastrointestinal, mammary, and central nervous system, indicating activation of proinflammatory signaling with increased persistent oxidative stress (108). These effects have been seen at the metabolomics level. For example, Cheema et al (109) reported that long-term perturbations in nucleic acid and amino acid metabolism were triggered by HZE radiation in the gastrointestinal tract.

Although both low LET and high LET radiation affected amino acid metabolism, high LET radiation preferentially altered dipeptide metabolism and caused upregulation of "prostanoid biosynthesis" and "eicosanoid signaling," which are interlinked events related to cellular inflammation and have implications for nutrient absorption and inflammatory bowel disease during space missions and after RT. Taken together, these initial findings indicate that one would expect differential effects of carbon ion therapy on aspects of metabolism in both normal and tumor tissues. Biomarkers at the metabolomics levels in biofluids might have utility in assessing responses to particle ion therapy and understanding the effect on cellular metabolism.

In summary, significant fundamental questions exist for which omics analysis can provide useful information regarding the responses of cells and tissues to charged particles. Furthermore, analysis of specimens from clinical trials, in which omics analysis has been integrated into the trial design, is critical for charged particle therapy, and for radiation therapy in general.

Tumor (Human) RBE

With regard to the tumor RBE of protons, low LET proton radiation induces slightly more complex DNA damage than reference photon radiation, resulting in an average RBE of ~1.1 (110). However, in vitro evidence is now accumulating that alterations in homologous recombination repair (HRR) and Fanconi anemia (FA) pathways sensitize normal and cancer cells to proton radiation, leading to RBE values of $\geq$1.3 when cells are irradiated at the mid-spread out Bragg peak (111-114). Together, these observations support a model in which HRR/FA defects impair a cell's ability to repair and restart DNA replication forks that encounter proton-induced clustered DNA damage (112, 114). The clinical significance of these data is that alterations in HRR/FA can be found in tumor types that increasingly are treated with protons, including, for example, prostate and lung cancer (115, 116). Furthermore, because most, if not all, human tumors harbor defects in $\geq$1 DNA damage response (DDR) pathways, non-HRR/FA defects might exist that would also lead to an elevated proton RBE. Unbiased screens of tumor cell lines and fresh human tumor biopsy specimens and of DDR disruptions would be needed to address this possibility and, furthermore, to establish the incidence and range of RBE variations that exist. Additional studies are also needed to confirm these RBE values in vivo, for example, through appropriate xenograft experiments using the tumor control probability as an endpoint. Genomic biomarkers or functional DDR foci biomarkers will be required to identify tumors with a RBE of >1.1 (112, 117). These biomarkers warrant correlation with clinical outcomes in ongoing randomized proton/photon studies, for example in prostate and lung cancer. This novel paradigm of a variable tumor RBE yields several potential therapeutic opportunities: (*1*) physical dose de-escalation to reduce toxicity, for example, in pediatric patients; (*2*) combination with targeted anticancer agents, such as DNA repair inhibitors or immune checkpoint inhibitors; (*3*) the use of scanned beams to increase the LET in the tumor to synergize with the underlying biological RBE advantage; and (*4*) the selection of patients for proton treatment slots that otherwise would not have been available to them.

With regard to tumor RBEs for charged particles heavier than protons, the pioneering research of Blakely et al (118) and Blakely and Chang (119) suggested that, based on the ratio of peak to plateau biologically effective doses for several charged particles, the "optimal" ion for therapy was carbon, although ions heavier than carbon would have better oxygen gain factors. The findings were validated by teams from Japan and Germany (29, 31); however, relatively few studies used human tumor cells. Historically, an RBE of ~3 has been assumed for carbon ions, although even just for human tumor cells in vitro, the carbon RBEs can range from 1 to 5 (110). Furthermore, other than carbon ions, few ions between helium and oxygen have been assessed systematically for RBEs. Because it has been recognized that RBE values depend on many factors, including cell type, radiation dose, dose rate, particle type, and energy, a need remains for quantitative data on RBEs for a variety of tumors and normal tissues exposed to a range of ions. Additionally, such information is vital for integration into biophysical models for treatment planning. Preliminary studies using human tumor cell lines exposed to various ion species at several positions along a pristine Bragg peak (Held et al, unpublished data) suggest that at the Bragg peak, RBE values for helium ions approach those for carbon ions with some cell lines. This effectiveness of helium was indicated in early data from Blakely et al and is consistent with a recent analysis of available data on human tumor cells (110) and with the clinical efficacy of helium ions in treating uveal melanoma (120). Important issues remaining to be addressed in RBE studies with heavier ions include the efficacy of ions other than carbon; experimental approaches in vitro and in vivo that are most effective to yield RBE information useful for translation to the clinic; magnitude of the therapeutic gain with ions (ie, relative magnitude of RBEs for tumors vs critical normal tissues); and the possible role of defects in DNA repair in tumor cells that might help guide the selection of patients for ion therapy.

Hypoxia

Tumor cells lose the capacity to control cell division and the normal contact inhibition of growth when encountering neighboring cells (121). The tissue structure that results from this disorganized growth leads to a shapeless mass in which many cell regions cannot be reached by oxygen and nutrients. Tumors then require angiogenesis to provide a supply of nutrients; however, spatial and temporal inadequacies in the delivery of oxygen and other nutrients

can occur and might drive continued aberrant angiogenesis. Cycling hypoxia and free radicals regulate angiogenesis and radiation therapy responses (122). Hypoxic tumor cells are resistant to conventional photon RT (123). The anoxic (0% oxygen concentration) OER, the ratio between the dose at the hypoxic condition and the dose in the oxic condition to produce the same biological effect, is ~3.0 (124, 125). Charged-particle therapy can eradicate hypoxic tumor cells (66, 69, 94), depending on a number of physical and biological characteristics, including particle species, LET, absorbed dose, dose rate, track structure, treatment-targeting plan, dose-fractionation regimen (126-128), tumor microenvironment characteristics (129), influence of cancer stem cells (CSCs), cell-cycle phase (130), kinetics, antioxidants (130), metabolic state, expression of cytokines such as vascular endothelial growth factor, TGF-β, fibroblast growth factor (131), epithelial growth factor, transcription factors such as HIF-1/HIF-2) (132, 133), and overall treatment schedule.

The biology of hypoxia in tumors and normal tissues is complex and dynamic, and controversial data have been reported concerning the time frames between the acute and chronic hypoxic conditions and the resulting biological consequences (134). The profile of dose and LET differs in the Bragg peak of an ion beam relative to the entrance plateau. This differential of biologically effective dose must be recognized in the treatment planning of hypoxic tumors and the sparing of surrounding normal tissues (66). Modern 3-dimensional combined PET/computed tomography imaging can identify hypoxic tissues in real time (135). The linear-quadratic model has been suggested to be incorrect when used for hypofractionation (136). Dose hypofractionation with fewer, but larger, dose fractions is most effective against tumors with persistent hypoxia (36). However, the advantage is diminished for tumors with dynamic hypoxia owing to local changes in oxygenation (128,137-141). In vitro investigations of radiation sensitivity have consistently shown that cells irradiated under acute hypoxia are more radioresistant than cells irradiated under chronic hypoxia (134, 142). The best clinical gain is achieved with optimization of the treatment with respect to both the tumor and the normal tissue responses. Results have shown that clinically relevant RBE values increase with higher doses per fraction (127), in contrast to the results from single-dose experiments in which RBE values increase at low doses. Late-reacting tissues might be at a greater risk of high LET radiation damage than early-reacting tissues. These aspects could increase the therapeutic window for slow-growing tumors. For normal tissues, they seem to counterbalance the other potential advantages of high LET radiation that generally support the use of hypofractionated regimens for this type of radiation (7, 143, 144).

CSCs are thought to be responsible for tumor initiation, recurrence, metastasis formation, and resistance to any common cancer therapies, including RT (145). Furthermore, hypoxia is a critical microenvironmental factor in regulating the self-renewal of CSCs (145). HIF-2α seems to be an important gene and a key regulator of hypoxia in cells (146). CSCs have high HIF activity in normoxic environments, and HIF and vascular endothelial growth factor (147) activity is critical in the maintenance of CSCs (148). Targeting the hypoxic niches could increase treatment success. Compelling evidence has shown that CSCs contribute to cell proliferation, invasion, and metastasis (145). It is now possible with the research treatment planning system TRiP98, not only to optimize the biologically effective dose (RBE-weighted), but also, with the new extension TRiP98-OER, to account for differentially oxygenated regions and to plan the biologically isoeffective dose in the local tumor microenvironment. This accomplishes "kill-painting" of hypoxic tumors in charged particle therapy (32, 149). More basic biophysical research is needed to explore the full potential of particle dose regimens and to determine why they have been especially effective against radioresistant, slow-growing human tumors (110).

Multiple open questions remain. Can we optimize both biological and physical parameters for charged-particle therapy and how might these factors affect the clinical outcomes? How can we improve the imaging of hypoxic regions inside the tumor? Would it be enough to target the CSCs? What would be a useful marker for a CSC niche? Which particle is best for hypoxic tumors: carbon, oxygen, or combined/boost treatments with multiple ions? Do we fully understand the underlying mechanisms whereby particle radiation sensitizes the hypoxic region of the tumor? Which tumor sites would be best to demonstrate the full potential of particle therapy for radioresistant hypoxic tumors?

Carcinogenesis

In the United States, 6% to 10% of all cancers diagnosed are second malignant neoplasms (SMNs). Understanding the risks associated with charged particle radiation-induced SMNs compared with photon-induced risks is an important factor in defining the future of particle RT.

Currently, the SMN risks for patients treated with charged particle RT are unknown. Epidemiologic studies, by themselves, will not provide timely assessments of risks. Radiation-induced solid tumors can take decades to develop but changes to treatment planning and therapy equipment are continuous. Estimating the true incidence of radiation-induced SMNs is also difficult because the cancer risks associated with primary tumors such as smoking can also predispose an individual to developing second cancers. Genetic factors play a role in the susceptibility to SMNs and, other than prostate and cervical cancers, for which surgery alone can be an alternative treatment, no good control groups can be used as a reference. In particular, patients with childhood cancers could be at increased risk owing to their small body size, age at treatment, and greater exposure to scattered radiation owing to the small distance

between the treatment volume and nearby organs (150). Modeling has predicted the dose distributions from the primary fields for IMRT and proton therapy, and several investigators have reported that the risks of SMN are comparable or lower after particle RT (151, 152). Early reports of retrospective and prospective analyses of the risk of second malignancies suggests a reduced risk of second malignancies in patients receiving proton therapy, including small studies of childhood cancers. The risk of second malignancies was reviewed by Eaton et al (153). Probably the largest study that strictly examined second cancer risk is that by Chung et al (154). They performed an analysis of >500 matched protons versus photons patients treated at the Harvard cyclotron (1973-1992) with matched patients from the Surveillance, Epidemiology, and End Results database. They reported that proton therapy slightly reduced the risk of second malignancy compared with photons (154).

The SMN risks must be modeled, in part, on the results from animal studies. Although the dose-distribution characteristics of particle radiation offer the advantage of more precise treatment planning protocols, minimizing the dose to nontargeted tissues, the cumulative dose to the normal tissue can still be substantial. Of concern, high-charge, high-energy (HZE) ions have high RBEs (20-70) for some radiogenic solid tumors in rodent models. In contrast, RBEs for hematologic malignancies appear to be low (155-160). However, limitations exist with the available animal studies. Because they were mostly designed to answer questions about cancer risks to spaceflight crewmembers from galactic cosmic radiation exposures, the doses used are low compared with those used in RT, the ions and energies examined reflect those in the galactic cosmic radiation spectrum, and the exposures are usually to the whole body. Data on carcinogenesis dose responses for radiation qualities and doses relevant to particle therapy are sparse (161, 162). Also lacking are data on the effects of fractionation and partial body exposure relevant to particle therapy.

Among the open questions remaining is whether the SMN experience with low LET exposures can inform risk modeling for high LET exposures, specifically whether low LET and high LET exposures lead to the same types of tumors by the same mechanisms. Also of interest with the advent of next generation sequencing and the move toward personalized medicine is whether patients susceptible to SMNs can be identified and, if so, is it worth doing?

Owing to the length of time needed to accumulate epidemiologic data, a concerted multicenter, international effort should be established for long-term follow-up of charged particle RT patients. Animal studies to determine the carcinogenic efficacies of charged particles for radiation qualities and fractioned partial body exposure relevant to charged particle RT are needed. Animal studies and genetic epidemiologic studies designed to address the open questions should be undertaken.

NCI Request for Information for Particle Beam Facility for Cancer Research

Before convening the workshop, the NCI in December 2016 issued a request for information seeking input on a particle beam facility for RT-related cancer research (NOT-CA-16-011). The goal of this request for information was to gain feedback, comments, and novel ideas from members of cancer research communities regarding the need, characteristics, and potential utility of a facility for particle beam RT (PBRT)-related cancer research. Comments received from both industry and academic centers were discussed at the workshop. They included

- The main issues relevant to the clinical uses of PBRT that can be efficiently addressed in preclinical studies
- The types of research that might be designed to address such issues
- Particle beam species and properties required or desirable
- Optimal infrastructure of such a facility
- Estimated particle beam time for potential preclinical research

The responders identified the following topics of preclinical in vitro and animal research to be the most relevant for the successful implementation of PBRT in the United States:

- Reduction of uncertainties in dose calculation and delivery
- Development of dosimetric and imaging tools
- Treatment planning tools using the Monte Carlo method
- IMPT optimization that would allow dose painting and considering RBE, LET, and/or other relevant parameters
- Development of new methods for motion mitigation
- Microdosimetry and radiobiology studies of the mechanisms of particle therapy-induced damage to characterize the biological effect parameters that would allow comparison with well-known effects of conventional RT in various tissues and microenvironments, including hypoxia
- Investigations of the efficacy of different ion beams and optimization of their combinations with other therapeutic interventions, including radiosensitizers and immunotherapy
- Identification of relevant biomarkers, including radiogenomics
- The development of relevant animal models
- The use of virtual clinical trials to define the appropriate indications and formulate hypotheses for clinical trials
- Investigations of the possible side effects of particle beam therapy, such as carcinogenesis, accelerated aging, and the shift of microbial community constituents

The following were proposed as required or desirable characteristics of the PBRT facility:

- Protons to carbon required and oxygen to silicon desirable

- Horizontal and vertical beams required; a gantry desirable
- Pencil beams with raster scanning capability
- Capability to accelerate light ions $\leq$500 MeV/u or range $>$30 cm
- Better than 1-mm scanning spot position precision
- Large fields 20 cm $\times$ 20 cm or $\sim$28 cm in diameter
- Dose delivery homogeneity in 10 $\times$ 10 $\times$ 10 cm^3 cube better than 3%
- Dose rate of $\geq$2 Gy/min
- Upgradability
- Absolute dosimetry traceable to a national standard
- Laboratories for histologic testing and cell culture (tissue embedding stations, microtomes, cryotomes, hoods, incubators, fluorescent and regular light microscopes, etc)
- Anesthesia unit for small and large animals
- Imaging facility for small and large animal computed tomography, magnetic resonance imaging, PET
- Laser-based alignment of phantoms, animals, and cell culture flasks
- Electronically controlled table with precise displacement in *x*-, *y*-, and *z*-dimensions and rotation
- Animal housing facility capable of accommodating mice, rats, rabbits, and minipigs with food, water, lighting (diurnally regulated), and a procedure room

These characteristics will be considered by the NCI in providing support for the establishment of the appropriate infrastructure for preclinical research.

Summary and Conclusions

RT with protons and heavier ions has been practiced now for decades. However, the number of patients treated with particle therapy has been very low compared with those treated with photons. Regardless, a resurgence in interest has occurred in particle therapy. Likely driven by cost factors, proton therapy relative to other particles is expanding most rapidly in clinical practice. Although heavy ion centers are more costly to develop, therapy with ions such as carbon might have distinct physical and biological advantages, especially for cancers historically deemed radiation resistant. They might also have advantages in terms of reduced immunosuppression and increased immunogenicity. However, our understanding of the unique biology of ion beams, even for protons, and its relationship to physical factors is limited, although rapidly advancing. To maximize the clinical potential of both light and heavier ion therapy, a great amount of research is needed to inform the treatment planning process. In contrast to such modifications as the addition of chemotherapy to RT regimens, altering fractionation schemes, and so forth, particle therapy is a fundamentally different form of radiation, one with great clinical potential. We hope that, based on the discussions at the workshop, the NCI will recognize this potential and develop research programs directed at the needed research.

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