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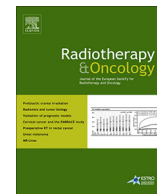
Review

Toward A variable RBE for proton beam therapy

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A R T I C L E I N F O

A B S T R A C T

In the clinic, proton beam therapy (PBT) is based on the use of a generic relative biological effectiveness (RBE) of 1.1 compared to photons in human cancers and normal tissues. However, the experimental basis for this RBE lacks any significant number of representative tumor models and clinically relevant end-points for dose-limiting organs at risk. It is now increasingly appreciated that much of the variations of treatment responses in cancers are due to inter-tumoral genomic heterogeneity. Indeed, recently it has been shown that defects in certain DNA repair pathways, which are found in subsets of many cancers, are associated with a RBE increase *in vitro*. However, there currently exist little *in vivo* or clinical data that confirm the existence of similarly increased RBE values in human cancers. Furthermore, evidence for variable RBE values for normal tissue toxicity has been sparse and conflicting to date. If we could predict variable RBE values in patients, we would be able to optimally use and personalize PBT. For example, predictive tumor biomarkers may facilitate selection of patients with proton-sensitive cancers previously ineligible for PBT. Dose de-escalation may be possible to reduce normal tissue toxicity, especially in pediatric patients. Knowledge of increased tumor RBE may allow us to develop biologically optimized therapies to enhance local control while RBE biomarkers for normal tissues could lead to a better understanding and prevention of unusual PBT-associated toxicity. Here, we will review experimental data on the repair of proton damage to DNA that impact both RBE values and biophysical modeling to predict RBE variations. Experimental approaches for studying proton sensitivity *in vitro* and *in vivo* will be reviewed as well and recent clinical findings discussed. Ultimately, therapeutically exploiting the understudied biological advantages of protons and developing approaches to limit treatment toxicity should fundamentally impact the clinical use of PBT.

Introduction

There exists a great potential for therapeutic benefits of proton beam therapy (PBT) in several cancer types [1]. PBT has superior physical characteristics compared to standard photon radiation in many anatomical sites, but its biological properties have been thought to be similar to photons [2,3]. This is reflected by the use of a generic relative biological effectiveness (RBE) of 1.1 for both cancer and normal tissues (Fig. 1). However, there exists a scarcity of data on RBE variations in human tumors. In a comprehensive review from 2002, the average RBE was estimated as ∼1.2 *in vitro* and ∼1.1 *in vivo*. However, most of the 20 cell lines were of Chinese hamster ovary (CHO) origin resulting in a somewhat higher *in vitro* RBE, and only 7 human cancer cell lines were included [2]. In a recent update, the number of cancer cell lines remained limited, with an average RBE of ∼1.15 [3]. However, there remains considerable variability related to both experimental conditions (incl. dose, beam characteristics) and cell biology (incl. DNA repair status, α/β ratio).

This experimental basis for the current clinical use of a generic RBE of 1.1 is a major limitation, given the considerable genomic heterogeneity across cancers even for the same type and histology as unraveled by recent genomic studies. Moreover, it is increasingly appreciated that much of the variations in treatment sensitivity observed clinically are due to inter-tumoral heterogeneity, which includes alterations in the DNA damage response (DDR) [4–9]. Indeed, several reports have now demonstrated that defects
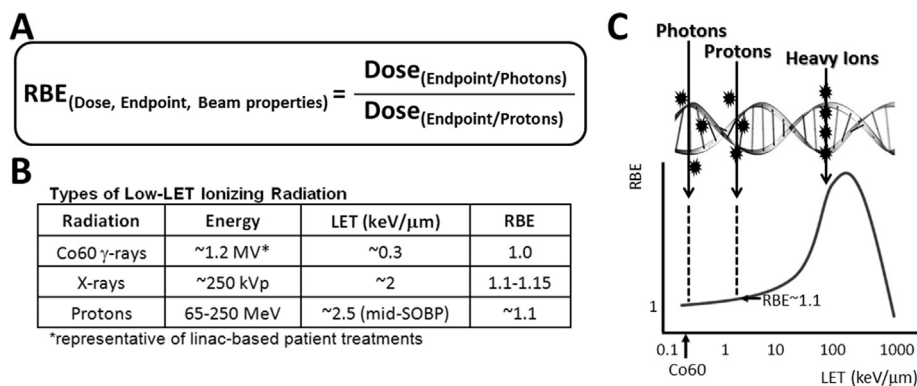
Fig. 1. Relative Biological Effectiveness (RBE) of different ionizing radiation qualities. (A) RBE is the ratio of doses to reach the same level of effect (Endpoint) when comparing two radiation modalities, i.e., reference photons (Cobalt-60, Co60) vs. protons. The radiation dose in PBT is prescribed as Gy[RBE] according to ICRU. For example, for a RBE = 1.1 and a desired Co60 equivalent (Co60Eq) dose of 2 Gy, the corresponding physical proton dose would be 1.82 Gy (1.82 Gy × 1.1 = 2 Gy[RBE]). (B) Examples of ionizing radiation types and representative dose-average LET (Linear Energy Transfer) and RBE values [10,103,104]. LET of γ-rays refers to secondary electrons. (C) Illustration of how protons induce DNA damage that is slightly more clustered than photons (or Co60 γ-rays), which in DNA repair-proficient cells yields a RBE of 1.1.

in the homologous recombination (HR) and Fanconi Anemia (FA) DDR pathways are associated with RBE values of 1.3 or more *in vitro* [10–13]. However, there currently exist few pre-clinical *in vivo* or clinical data to demonstrate the existence of increased RBE values in human cancers, and evidence with regard to RBE variations in normal tissues remains sparse as well [2,14–16].

Importantly, we lack an in-depth understanding of the mechanisms that underlie RBE variations in tumors and normal tissues, and we are currently unable to identify individual cancer patients whose tumors and/or normal tissues exhibit increased sensitivity to PBT. These shortcomings constitute critical barriers to fully harnessing the potential superiority of PBT and to avoiding unnecessary toxicity. Here, we review our current knowledge of and approaches to understanding RBE variations in tumors and normal tissues. In the not too distant future, therapeutically exploiting the understudied biological advantages of protons and developing approaches to limiting treatment toxicity are expected to fundamentally impact the clinical use of PBT in the increasing number of proton centers worldwide.

DNA damage caused by particle radiation and its repair

DNA repair of double-strand breaks and clustered damages

Although the use of charged particle therapy has increased rapidly over the last few decades, the contribution and the interplay of specific DNA repair pathways to the repair of DNA lesions induced by these radiation modalities is incompletely understood. Particle radiations such as proton or carbon ion beams induce more highly localized and clustered DNA damage than X- and γ-rays. Clustered DNA damage includes abasic sides, base damages, single- (SSBs) and double-strand breaks (DSBs) that are in close proximity to each other [17]. The complexity and yield of radiation-induced clustered DNA damage increases with ionization density [18–21]. Hence, for a given dose, therapeutic carbon beams (200–430 MeV/n; ~>10–80 keV/μm linear energy transfer (LET)) are expected to induce more clustered DNA lesions than therapeutic proton beams (65–250 MeV/n; ~2–10 keV/μm). Clustered DNA damage represents a considerable obstacle to efficient repair, and DSBs within clustered lesions rejoin with slower kinetics and less completely than frank DSBs [20,22], likely contributing to the observed higher RBEs for cell killing after charged particle- compared to photon-irradiated cells [18,23]. A major question is whether the mechanisms of repairing DNA damages caused by PBT resemble those triggered by photons or those operating in response to heavy ion exposure.

When potentially lethal DSBs occur, cells repair these DNA ends mainly by two distinct pathways, non-homologous end joining (NHEJ) and HR. These two pathways differ biochemically, have different substrate requirements, and are used differently throughout the cell cycle (for review, see [24]). Briefly, NHEJ is the main pathway of ionizing radiation-induced DSB repair in G1- and early S-phase cells while both HR and NHEJ contribute to DSB repair in late S-/G2-phase cells [25,26]. Importantly, HR also is the predominant pathway for the repair of stalled and damaged DNA replication forks [27,28]. Notably, mutations in HR genes increase cellular sensitivity to photon radiation and also to replicative and transcriptional stress [29,30]. During HR a DSB, or a DNA replication fork encountering a DNA lesion, undergoes nucleolytic resection to yield 3′ single-stranded (ss) DNA ends which are immediately covered by the ssDNA binding protein replication protein A (RPA). RPA is then replaced by the RAD51 recombinase forming a nucleoprotein complex termed the presynaptic filament. The presynaptic filament searches for, engages, and invades a homologous duplex target DNA to form the displacement loop (D-loop). DNA synthesis and resolution of DNA intermediates follows to complete HR repair [31]. During NHEJ, the KU70/80 heterodimer, which has high affinity for free DNA ends [13], initiates the pathway, whereby nucleolytic processing of DNA ends is blocked. KU70/80 recruit DNA-PKcs, and DNA-PKcs immobilizes the two DNA ends and facilitates the rejoining reaction [32–34], in which ligation is carried out by the XRCC4-DNA ligase IV complex [35]. NHEJ is the major repair pathway for DSBs induced by photon radiation including X-rays (for review, see [36]).

Repair pathways for high-LET radiations

To date, only a limited number of studies have addressed the relative contributions of NHEJ, HR and resection-mediated repair pathways to removing complex DSBs induced by different charged particle radiation types. Evidence is accumulating that shows that NHEJ is less capable of removing clustered DSBs induced by high-LET radiations as compared to low-LET radiations [37–42]. Yajima *et al.* [42] investigated the propensity of human and mouse cells to undergo DNA resection after low-LET (X- or γ-rays) versus high-LET radiations (70 keV/μm carbon (290 MeV/n) or 250 keV/μm iron ions (500 MeV/n)). Their study showed that >80% of the DSBs induced by heavy ions were subjected to end resection, which is significantly more than what was observed after low-LET radiations [41,43]. Interestingly, Yajima *et al.* [42] also reported on DNA resection occurring in G1 phase cells after heavy ion treatment and suggested that microhomology-mediated end-joining

(MMEJ) may take over from inefficiently working NHEJ at clustered DSBs in G1-phase cells. These findings are in accord with results reported by Averbeck and collaborators [44], who investigated G1-phase cells and a large number of heavy ion beams including 90 and 170 keV/μm carbon ions. These investigators also concluded that an increased requirement for processing of DNA ends at complex DSBs forces DNA repair pathway choice in G1-phase cells toward resection-dependent MMEJ repair [44].

One of the first studies that directly investigated the involvement of the HR pathway in repairing complex DSBs induced by heavy ions (1 GeV/n iron ions; LET = 150 keV/μm) compared the response to iron ions of HR-proficient and deficient Chinese hamster ovary (CHO) cell lines, and of syngeneic human cells depleted for the key HR protein RAD51 or its paralog RAD51D by RNA interference [45]. HR capability after iron ion irradiation was found to contribute to cell survival in rodent cells and to limiting mutagenesis in human cells, whereby the requirement for an intact HR pathway in protecting cells from the detrimental effects of charged particle radiation was demonstrated [45]. Interestingly, when the relative contributions of the NHEJ and HR pathways to the repair of DNA lesions induced by 200 MeV protons (energy spread at the mid-SOBP = 0–60 MeV; dose-average LET = 2.2 keV/μm), 290 MeV/n carbon ions (energy spread at the mid-SOBP = 0–160 MeV; dose-average LET = 50 keV/μm) or γ-rays were compared in CHO cell lines, HR capability was found to be more critical for the repair of carbon ion-induced DSBs than for the repair of DSBs induced by protons or by γ-rays [46].

The effects of a range of different heavy ions, including a 290 MeV/n carbon ion SOBP beam (LET = 50 keV/μm), on the survival of mouse embryonic fibroblasts (MEFs), fully DSB repair proficient or compromised in NHEJ or HR by homozygous deletion of the *Lig4* or *Rad54* genes, respectively, showed that defects in NHEJ lead to higher cellular sensitivity to therapeutic carbon beams than defects in HR [47]. However, it is interesting to note that, when compared to 200 kVp X-rays, RBE values for SOBP carbon ions at 10% survival were actually higher for *Rad54*-deficient MEFs (~2.1) than for *Lig4*-deficient MEFs (~1.4) [47]. These data suggest that, similar to the findings by Gerelchuluun *et al.* [46], even though NHEJ-deficiency is linked to the greatest cytotoxicity for all radiation types tested, exposure of HR-deficient cells to carbon ions is still more effective in reducing cell survival than exposure of HR-deficient cells to X-rays.

Repair pathways for low-LET protons

Grosse *et al.* reported that the removal of proton-induced DSBs preferentially relied on HR when comparing the cytotoxicity of 200 kVp X-rays to protons (energy spread at the mid SOBP ≤138 MeV) in CHO cells [13]. This study also showed that the DSB repair kinetics in HR-deficient cell lines were significantly delayed after proton irradiation compared to X-rays, and the authors concluded that PBT may be particularly beneficial to cancer patients with mutations in HR pathway genes. In a follow-up study, Fontana and collaborators investigated NHEJ and HR pathway choice in human A549 lung cancer cells depleted for RAD51 and in BRCA2-deficient ovarian carcinoma cells after exposure to X-rays and protons [12]. In accord with the results from their earlier study [13], an enhanced susceptibility of HR-deficient tumor cells to protons and an increased sensitivity of photon-irradiated tumor cells to NHEJ inhibitors were detected [12], further supporting the notion that tumors with defects in HR may be more susceptible to PBT than to photons. Similarly, data based on monitoring RAD51 and 53BP1 foci formation by stimulated emission depletion (STED) microscopy in S/G2-phase HeLa cells exposed to 21 MeV protons (LET = 2.56 keV/μm) showed that the HR pathway may be operating at higher levels after protons [48] than after photons [41].

An open question remained as to whether HR repair of DSB in late S/G2-phase or DNA replication fork-associated HR was required for the repair of proton-induced DNA damage. In a screen of 17 human lung cancer cell lines exposed to a clinical proton beam at mid-SOBP (235 MeV; 2.5 keV/μm), a range of proton RBE values was observed. Increased RBE values in the lung cancer and other human cell lines correlated with defects in HR or FA genes [49]. Importantly, HR and the FA pathway cooperate specifically at DNA replication forks to ensure fork protection and restart [28,50]. These data, therefore, suggested that clustered DNA damage resulting from proton irradiation challenges the progression of DNA replication forks in S-phase cells, leading to increased dependency on the HR/FA pathways for DNA damage repair and replication fork restart [49,51]. Liu and coworkers [11] corroborated this presumption demonstrating that immortalized human fibroblasts derived from a FA complementation group P patient with bi-allelic *SLX4* mutations are hypersensitive to protons, and that this hypersensitivity was linked to the SLX4-interacting MUS81 protein. Notably, both SLX4 and MUS81 are required for replication fork restart and HR (for review, see [28]).

In contrast to these studies [10–13], the report by Gerelchuluun et al. did not find an increased RBE of protons compared to X-rays when HR was impaired due to loss of XRCC2 or XRCC3 function [46]. The reason for this discrepancy remains unclear but may relate to assay parameters. Gerelchuluun et al. employed immediate plating for colony formation after irradiation which was not used in the other studies. It is possible that early trypsinization interferes with cell cycle progression and reduces the odds of DNA replication forks encountering unrepaired DSBs. Taken together, the majority of the published data suggest that the differential DNA repair capabilities between tumor and surrounding normal tissues could be exploited to improve regimens of PBT.

Since genetic or epigenetic alterations in HR and FA genes are increasingly being detected in many different cancer types [52–54], more cancer patients than historically thought may be appropriate candidates for PBT. Conceivably, other DDR defects and tumor genotypes may also be associated with increased RBE values. More *in vivo* and clinical data are urgently needed to substantiate the promising data obtained in cell culture experiments. It will also be necessary to more systematically investigate the link between HR/FA repair deficiencies and carbon ions, to improve our understanding of the similarities and differences of clustered DNA lesions induced by protons vs carbon ions. Ideally, a larger set of genomically characterized human cancer cell lines should be included in these experiments, as it has been done for protons in the past [49]. A model to depict the effects of different radiation qualities combined with deficiencies in the HR/FA pathways on RBE values is shown in Fig. 2.

Modeling and predicting the RBE of proton beam therapy

Even if we could determine the effects of DNA repair deficiencies that influence proton sensitivity in tumors, this may not be enough to predict clinically relevant RBE variations. Such predictions require quantitative models to assess how physical factors, such as radiation dose and LET, and biological parameters, such as tumor type and functional DNA repair status, combine within an individual's treatment. However, despite the growing understanding of the mechanistic drivers of RBE as described above and elsewhere [3], few biophysical models fully incorporate this knowledge, but instead fall into two broad categories.

Firstly, a number of models use conventional linear-quadratic dose responses, and modify the α and β parameters with LET-dependent terms to incorporate increasing damage complexity [55–57]. A second group of models incorporate increased physical
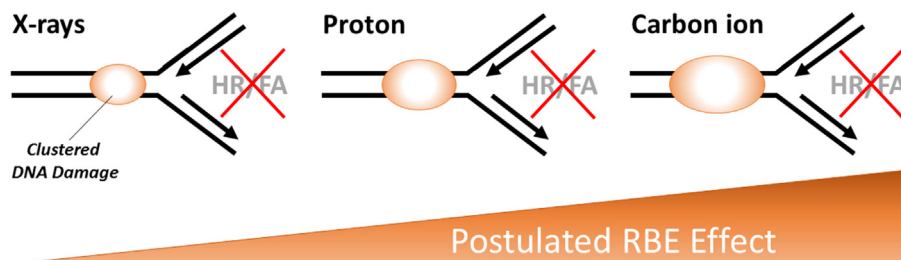
Fig. 2. Model: HR/FA defects lead to an increase in RBE as a function of clustered damage complexity. Cells with defects in DNA repair by homologous recombination (HR) or the Fanconi Anemia (FA) pathway are impaired in the ability to repair DNA replication forks that encounter clustered DNA damages. Even though the average LET is similar between orthovoltage X-rays and protons at mid-SOBP, protons are hypothesized to generate more complex lesions that present a greater obstacle to HR/FA-deficient cells than those produced by X-rays [10]. Clustered damages after high-LET carbon ion beam irradiation likely rely on an intact HR pathway for removal as well.

detail by replacing averaged LET with detailed sub-cellular dose calculations, including the Microdosimetric Kinetic Model [58], Local Effect Model [59], and Monte Carlo Damage Simulation [60]. A common feature of these models is that they do not incorporate significant detail about the underlying biology, typically extrapolating empirical parameters by fitting between photon and proton exposures. As a result, they have limited ability to incorporate our knowledge of the impact of genomic heterogeneity, in particular aspects of genomically determined radiation sensitivity. Consequently, new models are needed if inter-patient RBE heterogeneity is to be successfully incorporated into treatment planning.

Numerous models of radiation response processes are under development, including biophysical models of radiation-induced DNA damage distribution and complexity, models of DSB repair incorporating kinetics of protein recruitment and damage complexity, and models of cell death following different stresses [61–67]. But while these models can provide useful insights, most focus on a single endpoint, making them unsuitable to fully describe the variations in RBE which depend on multiple physical and biological factors. Integrated multi-scale models are needed to enable us to incorporate our knowledge of these processes to deliver individualized RBE predictions.

Some groups have begun to develop links between these different scales, including recent developments in the Local Effect Model, Giant Loop Binary Lesion Model, PARTRAC, and others [68–73]. These models have helped to compare which factors may be most significant in driving variations in RBE, and they have demonstrated the benefits of combining these factors into integrated models. However, the development of such models is challenging as each additional process to be modeled requires additional parameters which must be characterized and quantified, introducing uncertainties which are impossible to address through studies of cell survival based RBE values alone.

Development of multi-scale models instead demands close collaboration between experimentalists and modelers to design multi-scale experiments, combining terminal endpoints such as survival with quantification of intermediate processes such as DNA repair and other endpoints such as mutation or chromosome aberration formation to allow all stages of cellular radiation response to be characterized. By integrating all aspects of radiation response in individual studies, inter-experimental uncertainties can be reduced, enabling the development of more robust and extensible individualized RBE models.

Experimental approaches for determining proton RBE in vitro and in vivo

Although the number of operating proton therapy centers worldwide has been increased to more than 60, with currently 26 in the United States (www.ptcog.ch), it is important to realize that access to those centers for biological *in vitro* and *in vivo* experiments will likely be limited. This is not reflecting a lack of interest in proton research but rather the economical need for a financial return of an expensive treatment facility as well as the paucity of suitable laboratory space and animal facilities close by.

At the same time, there is a great need for more comprehensive and systematic RBE studies. The increased RBE in the distal fall-off of the Bragg-peak holds the potential for risks, i.e. potentially increased side effects due to uncertain RBE, as well as benefits, i.e. potentially enhanced tumor local control rates if they can be exploited through the use of intensity-modulated proton beam therapy. Additionally, RBE values in some tumors may be increased due to genomic factors as discussed above. Treatment planners will tackle dose distributions and range uncertainties, but pre-clinical research will need to shed light onto proton-specific normal tissue complications and tumor sensitivities. These findings should support patient stratification and guide a clinician's decision whether or not PBT is favored in an individual patient. Therefore, a proton-specific experimental pipeline is needed to support the translation of pre-clinical findings into clinically useful knowledge.

Recently, a pipeline for pre-clinical studies of radiation/drug combinations was proposed, emphasizing the importance of the *in vitro* clonogenic survival assay, medium/high-throughput assays for drug screening, physiological 3-dimensional (3D) assays as well as growth delay and tumor control experiments *in vivo* [74]. All of these techniques and endpoints have similar relevance for proton research. Limited access to proton beam time, nonetheless, lowers the number of biological samples which can be exposed and directly influences the range of parameters that can be studied in combination with proton radiation, e.g. number of cell lines/models, time points, radiation doses, drugs in different concentrations. As a consequence, the question arises which biological models should be used for proton research. It is increasingly held that a small number of established cell lines are not representative of the heterogeneity of human cancers although controversy exists [75–77]. Established cell lines do have the advantage that they grow reliably, are often genomically characterized, and are functionally studied (including characterization of radiation sensitivity). Over 20 years ago, the NCI60 cell line panel comprising nine cancer entities was established to standardize cancer drug research. Similar platforms are being developed for radiation purposes [78,79]. Analogously, panels of annotated cancer and normal cell lines with clinical relevance for PBT would be very useful. Such cell lines should have known X-ray (+/− drug) sensitivities. Selective inclusion of responder and non-responder cell lines for proton experiments would be of great value for mechanistical insight and for translational approaches as they might be indicative for clinical patient selection. Primary cell cultures preferentially grown as 3D models would be useful to confirm findings. Especially patient-derived cultures such as tumor or normal tissue organoids could help to stratify patients in the future if a correlation of results
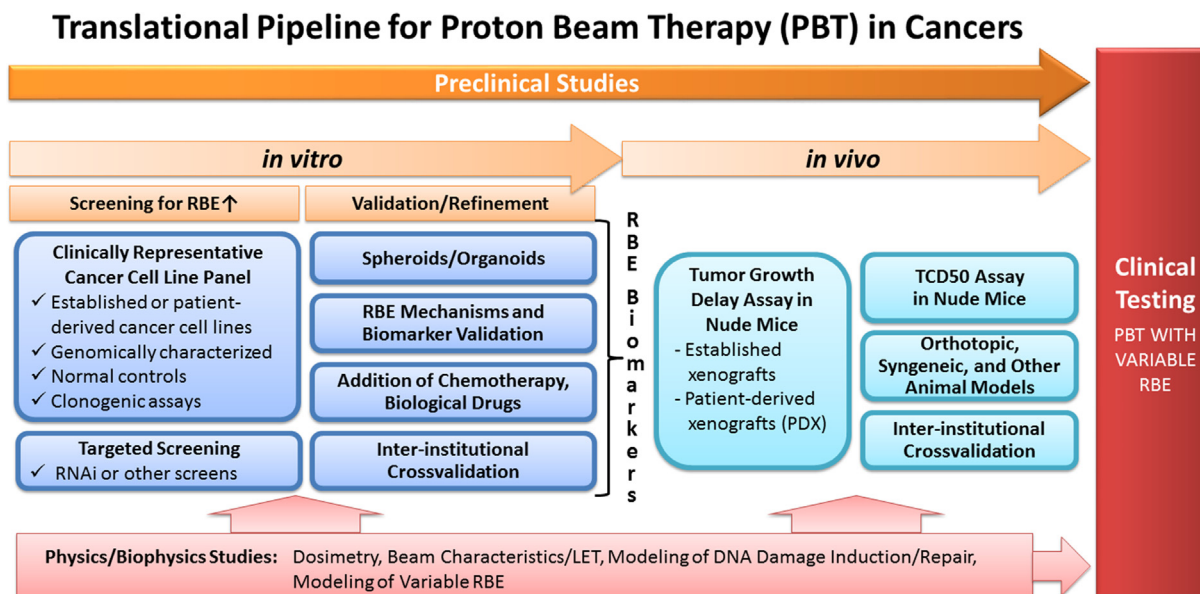
Translational Pipeline for Proton Beam Therapy (PBT) in Cancers

Fig. 3. Proposed translational pipeline for discovering clinically useful biomarkers that predict variable RBE values. See text for details.

from *in vitro* organoid cultures and patients' treatment outcomes could be established.

Performing *in vivo* experiments at clinical or experimental proton beam lines poses further challenges, including positioning of the animal and dose description for a small field [80]. For normal tissue endpoints, the RBE of protons is generally in the order of ~1.1 as reviewed [2,81]. However, much of these data pertain to skin reactions, and clinically significant endpoints such as involving the central nervous system or heart remain poorly studied. For tumor models, there exists a paucity of data. The tumor growth delay for a subcutaneous FaDu xenograft model following pulsed and continuous proton beams in comparison to 6 MV photons led to RBE values of 1.22 and 1.10, respectively [82]. In a Hep3B hepatoma xenograft model treated with 3 daily doses of 3 Gy 230 MeV protons at mid-SOBP or 6 MV photons, proton treatment resulted in 1.4-fold greater tumor growth delay which was statistically significant. Moreover, the *in vivo* effect mirrored the increased proton sensitivity seen in an *in vitro* colony formation assay, where the RBE at 0.5 survival fraction was around 1.5 (estimated from Fig. 2c in Ref. [82]). The authors also demonstrated that the difference in tumor effect between protons and photons could be pharmacologically enhanced. More *in vivo* data are urgently needed to derive *in vivo* RBE estimates and identify molecular targets for proton-specific radiosensitization.

Experience in photon research highlights the enormous relevance of tumor xenograft models for clinical translation [83–85]. Particularly, local tumor control dose experiments have demonstrated great potential relative to tumor growth delay in translating knowledge to the clinic [86,87]. However, subcutaneously transplanted tumors reach the desired tumor volume with temporal variations. Given the most often strictly defined days of proton beam time, loss of a large number of animals due to tumors of undesirable size faces ethical and biostatistical concerns. Furthermore, clinically relevant fractionation schedules, such as 30 fractions in 6 weeks, need to be adapted to proton beam availability. Here, shorter, hypofractionated schedules may be a viable and clinically relevant option. Still, local tumor control (TCD$_{50}$) experiments require approximately 80–100 animals per treatment arm, which essentially limits these studies to proton centers with dedicated rooms for experimental purposes.

To conclude, the establishment of a proton-adapted NCI60-like cancer cell line panel would be desirable to study mechanistic RBE effects, especially in combination with targeted drugs (Fig. 3). Patient-derived 3D/organoid cultures should be used for validation of results and, if a correlation with patient-outcome can be shown, may guide patient stratifications in the future. Clinically relevant *in vivo* experiments will be the bottle neck for the translational chain. These studies should consider not only relevant tumor models but also normal tissue endpoints, LET, and fractionation and thus may be best performed in centers with dedicated experimental rooms.

Toward clinical evidence for a variable proton RBE

In the clinical practice of heavy ion beam therapy, biologic effect models are explicitly considered in the treatment planning process in order to limit high-LET depositions in critical normal tissues. While the models used for treatment planning may differ between centers, with different models being used in Asia and Europe, variable RBE is incorporated in each. This has not been the case for PBT where it has been assumed that the RBE of protons is a constant value of 1.1 [2]. Around the world there has been a rapid expansion in the number of proton centers. As the number of patients treated with PBT increases, there is growing debate regarding the RBE of PBT [51,88–91].

In the laboratory, recent evidence indicates that the capacity for proton beams to cause biological damage is substantially higher near the distal, high-LET region [81,92–94]. This is true both for tumors as well as normal tissues. This is, of course, well known for heavy ions, but as yet not explicitly incorporated into proton treatment planning. Arguments most often made in support of a continued use of an RBE of 1.1 include that relative to heavy ions, the areas of high LET within a proton beam are very small and likely clinically insignificant if not within a critical organ at risk, and that there are no clinical data to suggest that the proton RBE is not 1.1 in most patients.

Increasingly, however, investigators are challenging the use of a generic RBE value of 1.1 for PBT using laboratory and clinical data. There are now several studies which have documented increased cell kill in the distal regions of proton beams, which

is supported by the observation of differential DNA damage along the beam path [92,93]. Clinically, the group at MD Anderson documented increased rates of post-radiation MR imaging changes in ependymoma patients treated with PBT compared to photons [14]. Such changes are indicative of early radiation injury and serve as an imaging biomarker of differential damage between these radiation types. In order to further evaluate the etiology of these changes, this group used Monte Carlo techniques to compute proton dose and LET distributions and found significant correlations between LET, dose, and regions of imaging change in these patients. Others are now expanding research in this area by conducting laboratory investigations, using normal tissue models, and clinical studies in expanded patient cohorts and other disease sites to better characterize the biologic effectiveness of PBT. Mining existing data bases and registries, especially at high-volume proton centers, may reveal associations between unusual biological effects and PBT (https://www.ptcog.ch/archive/patient_statistics/Patientstatistics-updateDec2016.pdf). For example, recent patient data indicate that for parenchymal lung changes induced by end-of-range protons the RBE is >1.1 [95]. On the other hand, no apparent increase was observed in brainstem necrosis in pediatric patients treated with PBT [96]. In contrast to a potentially variable tumor RBE, biological mechanisms underlying any RBE variations in normal tissues (other than low α/β values and LET increases at end-of-range) are essentially unknown at this time.

High level evidence for an increase in tumor RBE >1.1 in patients treated with PBT is also currently lacking. Because RBE increases may only exist in a subset of patients, such as the 20–25% of patients with HR/FA defects [97], associated local tumor control or survival effects are most likely missed when analyzing the study populations as a whole. Ideally, predictive biomarkers for tumor RBE >1.1 are needed to enrich a study population before randomizing patients to PBT vs photons in a clinical trial. Searches for a clinical signal of increased proton sensitivity and unusual responders in the randomized trials of PBT vs photons completed or soon to be completed will be of great interest [98] (NCT01512589, NCT01893307).

As the amount of data regarding a role for variable RBE increases, it seems that the field of PBT may follow that of heavy ions, with assessments of biological effectiveness incorporated into the treatment planning process. With newer proton therapy delivery modalities, in particular spot scanning proton therapy, intensity modulated proton therapy (IMPT) plans may be developed to preferentially divert high LET protons away from normal tissues into the target volume. This should reduce normal tissue damage while simultaneously increasing biologically effective doses to targets and perhaps even augmenting pre-existing RBE advantages due to certain DNA repair defects, further expanding the therapeutic index for PBT.

Conclusions

If we could predict variable RBE values in patients, we would be able to optimally use and personalize PBT. For example, such markers may facilitate selection of patients with proton-sensitive cancers if these patients would have been otherwise ineligible for PBT. Dose de-escalation may be possible to reduce normal tissue toxicity especially in pediatric patients. Knowledge of an increased tumor RBE may allow us to develop biologically optimized therapies to enhance local control, for example through optimized IMPT or specific drug combinations, while RBE biomarkers in normal tissues could lead to a better understanding and prevention of unusual PBT-associated toxicity. We also note that there continues to be a major international debate regarding the cost-effectiveness

of proton therapy [1,99–102]. Public, policy makers, and payers want to see evidence for the superiority of PBT and quantification of benefit, which to date has been framed only in technological terms. Realizing a biological advantage of PBT in subsets of cancer patients (and/or an increased risk for treatment toxicity in some patients), would fundamentally impact and redirect this debate.

Acknowledgements

Conflicts of interest

The authors declare that they have no conflict of interest.

References

[1] Durante M, Loeffler JS. Charged particles in radiation oncology. Nat Rev Clin Oncol 2010;7:37–43.

[2] Paganetti H, Niemierko A, Ancukiewicz M, Gerweck LE, Goitein M, Loeffler JS, et al. Relative biological effectiveness (RBE) values for proton beam therapy. Int J Radiat Oncol Biol Phys 2002;53:407–21.

[3] Paganetti H. Relative biological effectiveness (RBE) values for proton beam therapy Variations as a function of biological endpoint, dose, and linear energy transfer. Phys Med Biol 2014;59:R419–472.

[4] Garnett MJ, Edelman EJ, Heidorn SJ, Greenman CD, Dastur A, Lau KW, et al. Systematic identification of genomic markers of drug sensitivity in cancer cells. Nature 2012;483:570–5.

[5] Imielinski M, Berger AH, Hammerman PS, Hernandez B, Pugh TJ, Hodis E, et al. Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. Cell 2012;150:1107–20.

[6] Cancer_Genome_Atlas_Research_Network. Comprehensive genomic characterization of squamous cell lung cancers. Nature 2012;48:519–25.

[7] Pitroda SP, Pashtan IM, Logan HL, Budke B, Darga TE, Weichselbaum RR, Connell PP. DNA repair pathway gene expression score correlates with repair proficiency and tumor sensitivity to chemotherapy. Sci Transl Med 2014;6:229ra242.

[8] Kang J, D'Andrea AD, Kozono D. A DNA repair pathway-focused score for prediction of outcomes in ovarian cancer treated with platinum-based chemotherapy. J Natl Cancer Inst 2012;104:670–81.

[9] Iorio F, Knijnenburg TA, Vis DJ, Bignell GR, Menden MP, Schubert M, et al. A landscape of pharmacogenomic interactions in cancer. Cell 2016;166:740–54.

[10] Liu Q, Ghosh P, Magpayo N, Testa M, Tang S, Biggs P, et al. Lung cancer cell line screen links Fanconi Anemia/BRCA pathway defects to increased relative biological effectiveness of proton radiation. Int J Radiat Oncol Biol Phys 2015;91:1081–90.

[11] Liu Q, Underwood TS, Kung J, Wang M, Lu HM, Paganetti H, et al. Disruption of SLX4-MUS81 function increases the relative biological effectiveness of proton radiation. Int J Radiat Oncol Biol Phys 2016;95:78–85.

[12] Fontana AO, Augsburger MA, Grosse N, Guckenberger M, Lomax AJ, Sartori AA, et al. Differential DNA repair pathway choice in cancer cells after proton- and photon-irradiation. Radiother Oncol 2015;116:374–80.

[13] Grosse N, Fontana AO, Hug EB, Lomax A, Coray A, Augsburger M, et al. Deficiency in homologous recombination renders Mammalian cells more sensitive to proton versus photon irradiation. Int J Radiat Oncol Biol Phys 2014;88:175–81.

[14] Peeler CR, Mirkovic D, Titt U, Blanchard P, Gunther JR, Mahajan A, et al. Clinical evidence of variable proton biological effectiveness in pediatric patients treated for ependymoma. Radiother Oncol 2016;121:395–401.

[15] Giantsoudi D, Sethi RV, Yeap BY, Eaton BR, Ebb DH, Caruso PA, et al. Incidence of CNS injury for a cohort of 111 patients treated with proton therapy for medulloblastoma: LET and RBE associations for areas of injury. Int J Radiat Oncol Biol Phys 2016;95:287–96.

[16] Il Yu J, Choi C, Shin SW, Son A, Lee GH, Kim SY, et al. Valproic acid sensitizes hepatocellular carcinoma cells to proton therapy by suppressing NRF2 activation. Sci Rep 2017;7:14986.

[17] Asaithamby A, Chen DJ. Mechanism of cluster DNA damage repair in response to high-atomic number and energy particles radiation. Mutat Res 2011;711:87–99.

[18] Heilmann J, Taucher-Scholz G, Haberer T, Scholz M, Kraft G. Measurement of intracellular dna double-strand break induction and rejoining along the track of carbon and neon particle beams in water. Int J Radiat Oncol Biol Phys 1996;34:599–608.

[19] Goodhead DT. Energy deposition stochastics and track structure: what about the target? Radiat Prot Dosimetry 2006;122:3–15.

[20] Asaithamby A, Uematsu N, Chatterjee A, Story MD, Burma S, Chen DJ. Repair of HZE-particle-induced DNA double-strand breaks in normal human fibroblasts. Radiat Res 2008;169:437–46.

[21] Pastwa E, Neumann RD, Mezhevaya K, Winters TA. Repair of radiation-induced DNA double-strand breaks is dependent upon radiation quality and the structural complexity of double-strand breaks. Radiat Res 2003;159:251–61.

[22] Taucher-Scholz G, Heilmann J, Kraft G. Induction and rejoining of DNA double-strand breaks in CHO cells after heavy ion irradiation. Adv Space Res 1996;18:83–92.

[23] Hoglund H, Stenerlow B. Induction and rejoining of DNA double-strand breaks in normal human skin fibroblasts after exposure to radiation of different linear energy transfer: possible roles of track structure and chromatin organization. Radiat Res 2001;155:818–25.

[24] Mladenov E, Magin S, Soni A, Iliakis G. DNA double-strand break repair as determinant of cellular radiosensitivity to killing and target in radiation therapy. Front Oncol 2013;3:113.

[25] Hinz JM, Yamada NA, Salazar EP, Tebbs RS, Thompson LH. Influence of double-strand-break repair pathways on radiosensitivity throughout the cell cycle in CHO cells. DNA Repair (Amst) 2005;4:782–92.

[26] Rothkamm K, Kruger I, Thompson LH, Lobrich M. Pathways of DNA double-strand break repair during the mammalian cell cycle. Mol Cell Biol 2003;23:5706–15.

[27] Arnaudeau C, Lundin C, Helleday T. DNA double-strand breaks associated with replication forks are predominantly repaired by homologous recombination involving an exchange mechanism in mammalian cells. J Mol Biol 2001;307:1235–45.

[28] Pasero P, Vindigni A. Nucleases acting at stalled forks: how to reboot the replication program with a few shortcuts. Annu Rev Genet 2017;51:477–99.

[29] Branzei D, Foiani M. Maintaining genome stability at the replication fork. Nat Rev Mol Cell Biol 2010;11:208–19.

[30] Jasin M, Rothstein R. Repair of strand breaks by homologous recombination. Cold Spring Harb Perspect Biol 2013;5:a012740.

[31] Daley JM, Gaines WA, Kwon Y, Sung P. Regulation of DNA pairing in homologous recombination. Cold Spring Harb Perspect Biol 2014;6:a017954.

[32] Meek K, Gupta S, Ramsden DA, Lees-Miller SP. The DNA-dependent protein kinase: the director at the end. Immunol Rev 2004;200:132–41.

[33] Gottlieb TM, Jackson SP. The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen. Cell 1993;72:131–42.

[34] DeFazio LG, Stansel RM, Griffith JD, Chu G. Synapsis of DNA ends by DNA-dependent protein kinase. EMBO J 2002;21:3192–200.

[35] Davis AJ, Chen BP, Chen DJ. DNA-PK: a dynamic enzyme in a versatile DSB repair pathway. DNA Repair (Amst) 2014;17:21–9.

[36] Thompson LH. Recognition, signaling, and repair of DNA double-strand breaks produced by ionizing radiation in mammalian cells: the molecular choreography. Mutat Res 2012;751:158–246.

[37] Okayasu R, Okada M, Okabe A, Noguchi M, Takakura K, Takahashi S. Repair of DNA damage induced by accelerated heavy ions in mammalian cells proficient and deficient in the non-homologous end-joining pathway. Radiat Res 2006;165:59–67.

[38] Wang H, Wang X, Zhang P, Wang Y. The Ku-dependent non-homologous end-joining but not other repair pathway is inhibited by high linear energy transfer ionizing radiation. DNA Repair (Amst) 2008;7:725–33.

[39] Frankenberg-Schwager M, Gebauer A, Koppe C, Wolf H, Pralle E, Frankenberg D. Single-strand annealing, conservative homologous recombination, nonhomologous DNA end joining, and the cell cycle-dependent repair of DNA double-strand breaks induced by sparsely or densely ionizing radiation. Radiat Res 2009;171:265–73.

[40] Wang H, Zhang X, Wang P, Yu X, Essers J, Chen D, et al. Characteristics of DNA-binding proteins determine the biological sensitivity to high-linear energy transfer radiation. Nucleic Acids Res 2010;38:3245–51.

[41] Shibata A, Conrad S, Birraux J, Geuting V, Barton O, Ismail A, et al. Factors determining DNA double-strand break repair pathway choice in G2 phase. EMBO J 2011;30:1079–92.

[42] Yajima H, Fujisawa H, Nakajima NI, Hirakawa H, Jeggo PA, Okayasu R, et al. The complexity of DNA double strand breaks is a critical factor enhancing end-resection. DNA Repair (Amst) 2013;12:936–46.

[43] Beucher A, Birraux J, Tchouandong L, Barton O, Shibata A, Conrad S, et al. ATM and Artemis promote homologous recombination of radiation-induced DNA double-strand breaks in G2. Embo J 2009;28:3413–27.

[44] Averbeck NB, Ringel O, Herrlitz M, Jakob B, Durante M, Taucher-Scholz G. DNA end resection is needed for the repair of complex lesions in G1-phase human cells. Cell Cycle 2014;13:2509–16.

[45] Zafar F, Seidler SB, Kronenberg A, Schild D, Wiese C. Homologous recombination contributes to the repair of DNA double-strand breaks induced by high-energy iron ions. Radiat Res 2010;173:27–39.

[46] Gerelchuluun A, Manabe E, Ishikawa T, Sun L, Itoh K, Sakae T, et al. The major DNA repair pathway after both proton and carbon-ion radiation is NHEJ, but the HR pathway is more relevant in carbon ions. Radiat Res 2015;183:345–56.

[47] Takahashi A, Kubo M, Ma H, Nakagawa A, Yoshida Y, Isono M, et al. Nonhomologous end-joining repair plays a more important role than homologous recombination repair in defining radiosensitivity after exposure to high-LET radiation. Radiat Res 2014;182:338–44.

[48] Reindl J, Drexler GA, Girst S, Greubel C, Siebenwirth C, Drexler SE, et al. Nanoscopic exclusion between Rad51 and 53BP1 after ion irradiation in human HeLa cells. Phys Biol 2015;12:066005.

[49] Liu Q, Ghosh P, Magpayo N, Testa M, Tang S, Gheorghiu L, et al. Lung cancer cell line screen links fanconi anemia/BRCA pathway defects to increased relative biological effectiveness of proton radiation. Int J Radiat Oncol Biol Phys 2015;91:1081–9.

[50] Garcia-Higuera I, Taniguchi T, Ganesan S, Meyn MS, Timmers C, Hejna J, et al. Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. Mol Cell 2001;7:249–62.

[51] Held KD, Kawamura H, Kaminuma T, Paz AE, Yoshida Y, Liu Q, et al. Effects of charged particles on human tumor cells. Front Oncol 2016;6:23.

[52] Nepal M, Che R, Zhang J, Ma C, Fei P. Fanconi anemia signaling and cancer. Trends Cancer 2017;3:840–56.

[53] Riaz N, Blecua P, Lim RS, Shen R, Higginson DS, Weinhold N, et al. Pan-cancer analysis of bi-allelic alterations in homologous recombination DNA repair genes. Nat Commun 2017;8:857.

[54] Mutter RW, Riaz N, Ng CK, Delsite R, Piscuoglio S, Edelweiss M, et al. Bi-allelic alterations in DNA repair genes underpin homologous recombination DNA repair defects in breast cancer. J Pathol 2017;242:165–77.

[55] Carabe-Fernandez A, Dale RG, Jones B. The incorporation of the concept of minimum RBE (RbEmin) into the linear-quadratic model and the potential for improved radiobiological analysis of high-LET treatments. Int J Radiat Biol 2007;83:27–39.

[56] McNamara AL, Schuemann J, Paganetti H. A phenomenological relative biological effectiveness (RBE) model for proton therapy based on all published in vitro cell survival data. Phys Med Biol 2015;60:8399–416.

[57] Wedenberg M, Lind BK, Hardemark B. A model for the relative biological effectiveness of protons: the tissue specific parameter alpha/beta of photons is a predictor for the sensitivity to LET changes. Acta Oncol 2013;52:580–8.

[58] Hawkins RB. A microdosimetric-kinetic model for the effect of non-Poisson distribution of lethal lesions on the variation of RBE with LET. Radiat Res 2003;160:61–9.

[59] Elsasser T, Scholz M. Cluster effects within the local effect model. Radiat Res 2007;167:319–29.

[60] Frese MC, Yu VK, Stewart RD, Carlson DJ. A mechanism-based approach to predict the relative biological effectiveness of protons and carbon ions in radiation therapy. Int J Radiat Oncol Biol Phys 2012;83:442–50.

[61] Calzone L, Tournier L, Fourquet S, Thieffry D, Zhivotovsky B, Barillot E, et al. Mathematical modelling of cell-fate decision in response to death receptor engagement. PLoS Comput Biol 2010;6:e1000702.

[62] Ma L, Wagner J, Rice JJ, Hu W, Levine AJ, Stolovitzky GA. A plausible model for the digital response of p53 to DNA damage. Proc Natl Acad Sci U S A 2005;102:14266–71.

[63] McNamara A, Geng C, Turner R, Mendez JR, Perl J, Held K, et al. Validation of the radiobiology toolkit TOPAS-nBio in simple DNA geometries. Phys Med 2017;33:207–15.

[64] Meylan S, Incerti S, Karamitros M, Tang N, Bueno M, Clairand I, et al. Simulation of early DNA damage after the irradiation of a fibroblast cell nucleus using Geant4-DNA. Sci Rep 2017;7:11923.

[65] Nikjoo H, O'Neill P, Terrissol M, Goodhead DT. Quantitative modelling of DNA damage using Monte Carlo track structure method. Radiat Environ Biophys 1999;38:31–8.

[66] Schlatter R, Schmich K, Avalos Vizcarra I, Scheurich P, Sauter T, Borner C, et al. ON/OFF and beyond–a boolean model of apoptosis. PLoS Comput Biol 2009;5: e1000595.

[67] Taleei R, Nikjoo H. The non-homologous end-joining (NHEJ) pathway for the repair of DNA double-strand breaks: I. A mathematical model. Radiat Res 2013;179:530–9.

[68] Friedland W, Dingfelder M, Kundrat P, Jacob P. Track structures DNA targets and radiation effects in the biophysical Monte Carlo simulation code PARTRAC. Mutat Res 2011;711:28–40.

[69] Friedrich T, Durante M, Scholz M. Modeling cell survival after photon irradiation based on double-strand break clustering in megabase pair chromatin loops. Radiat Res 2012;178:385–94.

[70] Friedrich T, Scholz U, Elsasser T, Durante M, Scholz M. Calculation of the biological effects of ion beams based on the microscopic spatial damage distribution pattern. Int J Radiat Biol 2012;88:103–7.

[71] McMahon SJ, McNamara AL, Schuemann J, Paganetti H, Prise KM. A general mechanistic model enables predictions of the biological effectiveness of different qualities of radiation. Sci Rep 2017;7:10790.

[72] McMahon SJ, Schuemann J, Paganetti H, Prise KM. Mechanistic modelling of DNA repair and cellular survival following radiation-induced DNA damage. Sci Rep 2016;6:33290.

[73] Verkhovtsev A, Surdutovich E, Solov'yov AV. Multiscale approach predictions for biological outcomes in ion-beam cancer therapy. Sci Rep 2016;6:27654.

[74] Coleman CN, Higgins GS, Brown JM, Baumann M, Kirsch DG, Willers H, et al. Improving the predictive value of preclinical studies in support of radiotherapy clinical trials. Clin Cancer Res 2016;22:3138–47.

[75] Wilding JL, Bodmer WF. Cancer cell lines for drug discovery and development. Cancer Res 2014;74:2377–84.

[76] Pauli C, Hopkins BD, Prandi D, Shaw R, Fedrizzi T, Sboner A, et al. Personalized in vitro and in vivo cancer models to guide precision medicine. Cancer Discovery 2017;7:462–77.

[77] Ledford H. US cancer institute to overhaul tumour cell lines. Nature 2016;530:391.

[78] Abazeed ME, Adams DJ, Hurov KE, Tamayo P, Creighton CJ, Sonkin D, et al. Integrative radiogenomic profiling of squamous cell lung cancer. Cancer Res 2013;73:6289–98.

[79] Liu Q, Wang M, Kern AM, Khaled S, Han J, Yeap BY, et al. Adapting a drug screening platform to discover associations of molecular targeted radiosensitizers with genomic biomarkers. Mol Cancer Res 2015;13:713–20.

[80] Lühr A, Von Neubeck C, Krause M, Troost EGC. Relative biological effectiveness in proton beam therapy – Current knowledge and future challenges. Clin Transl Radiat Oncol 2018;9:35–41.

[81] Sorensen BS, Bassler N, Nielsen S, Horsman MR, Grzanka L, Spejlborg H, et al. Relative biological effectiveness (RBE) and distal edge effects of proton radiation on early damage in vivo. Acta Oncol 2017;56:1387–91.

[82] Zlobinskaya O, Siebenwirth C, Greubel C, Hable V, Hertenberger R, Humble N, et al. The effects of ultra-high dose rate proton irradiation on growth delay in the treatment of human tumor xenografts in nude mice. Radiat Res 2014;181:177–83.

[83] Gurtner K, Deuse Y, Butof R, Schaal K, Eicheler W, Oertel R, et al. Diverse effects of combined radiotherapy and EGFR inhibition with antibodies or TK inhibitors on local tumour control and correlation with EGFR gene expression. Radiother Oncol 2011;99:323–30.

[84] Koi L, Lock S, Linge A, Thurow C, Hering S, Baumann M, et al. EGFR-amplification plus gene expression profiling predicts response to combined radiotherapy with EGFR-inhibition: a preclinical trial in 10 HNSCC-tumour-xenograft models. Radiother Oncol 2017;124:496–503.

[85] Schutze C, Bergmann R, Bruchner K, Mosch B, Yaromina A, Zips D, et al. Effect of [(18)F]FMISO stratified dose-escalation on local control in FaDu hSCC in nude mice. Radiother Oncol 2014;111:81–7.

[86] Butof R, Dubrovska A, Baumann M. Clinical perspectives of cancer stem cell research in radiation oncology. Radiother Oncol 2013;108:388–96.

[87] Krause M, Zips D, Thames HD, Kummermehr J, Baumann M. Preclinical evaluation of molecular-targeted anticancer agents for radiotherapy. Radiother Oncol 2006;80:112–22.

[88] Paganetti H. Relating proton treatments to photon treatments via the relative biological effectiveness-should we revise current clinical practice? Int J Radiat Oncol Biol Phys 2015;91:892–4.

[89] Mohan R, Held KD, Story MD, Grosshans D, Capala J. Proceedings of the national cancer institute workshop on charged particle radiobiology. Int J Radiat Oncol Biol Phys 2018;100:816–31.

[90] Mohan R, Peeler CR, Guan F, Bronk L, Cao W, Grosshans DR. Radiobiological issues in proton therapy. Acta Oncol 2017;56:1367–73.

[91] Jones B, McMahon SJ, Prise KM. The radiobiology of proton therapy: challenges and opportunities around relative biological effectiveness. Clin Oncol (R Coll Radiol) 2018.

[92] Cuaron JJ, Chang C, Lovelock M, Higginson DS, Mah D, Cahlon O, et al. Exponential increase in relative biological effectiveness along distal edge of a proton bragg peak as measured by deoxyribonucleic acid double-strand breaks. Int J Radiat Oncol Biol Phys 2016;95:62–9.

[93] Chaudhary P, Marshall TI, Currell FJ, Kacperek A, Schettino G, Prise KM. Variations in the processing of DNA double-strand breaks along 60-MeV therapeutic proton beams. Int J Radiat Oncol Biol Phys 2016;95:86–94.

[94] Saager M, Peschke P, Brons S, Debus J, Karger CP. Determination of the proton RBE in the rat spinal cord: is there an increase towards the end of the spread-out Bragg peak? Radiother Oncol 2018.

[95] Underwood TS, Grassberger C, Bass R, Jimenez R, Meyersohn N, Yeap BY, et al. Clinical evidence that end-of-range proton RBE exceeds 1.1: lung density changes following chest RT. Radiother Oncol 2017;123:S123–4.

[96] Gentile MS, Yeap BY, Paganetti H, Goebel CP, Gaudet DE, Gallotto SL, et al. Brainstem injury in pediatric patients with posterior fossa tumors treated with proton beam therapy and associated dosimetric factors. Int J Radiat Oncol Biol Phys 2018;100:719–29.

[97] Willers H, Pfäffle HN, Zou L. Targeting homologous recombination repair in cancer. Elsevier: Academic Press; 2012.

[98] Chang JY, Verma V, Li M, Zhang W, Komaki R, Lu C, et al. Proton beam radiotherapy and concurrent chemotherapy for unresectable stage III non-small cell lung cancer: final results of a phase 2 study. JAMA Oncol 2017;3:e172032.

[99] Macready N. The promise of protons in cancer therapy. J Natl Cancer Inst 2012;104:648–9.

[100] Mitin T, Zietman AL. Promise and pitfalls of heavy-particle therapy. J Clin Oncol 2014;32:2855–63.

[101] Bortfeld TR, Loeffler JS. Three ways to make proton therapy affordable. Nature 2017;549:451–3.

[102] Mohan R, Grosshans D. Proton therapy – Present and future. Adv Drug Deliv Rev 2017;109:26–44.

[103] Hall EJ. The relative biological efficiency of x rays generated at 220 kVp and gamma radiation from a cobalt 60 therapy unit. Br J Radiol 1961;34:313–7.

[104] Spadinger I, Palcic B. The relative biological effectiveness of 60Co gamma-rays, 55 kVp X-rays, 250 kVp X-rays, and 11 MeV electrons at low doses. Int J Radiat Biol 1992;61:345–53.