Clinical Oncology xxx (2018) 1–4

Contents lists available at ScienceDirect

Clinical Oncology

journal homepage: www.clinicaloncologyonline.net

Science in Focus: Biological Optimisation of Radiotherapy Fraction Size in an Era of Immune Oncology

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Received 30 April 2018; accepted 28 June 2018

Detailed genomic characterisation of tumours reported by consortiums such as The Cancer Genome Atlas and the International Cancer Gene Consortium has established that extensive inter-tumoural heterogeneity exists between patients within tumour sites [1–4]. Radiotherapy fractionation is currently delivered as a 'one size fits all' approach, with uniform fractionation within each organ site. However, the inter-tumoural variation described above indicates that there is potential to individualise fractionation within tumour sites to maximise therapeutic gain. Realisation of such potential necessitates understanding the molecular biology underlying sensitivity to fraction size. This article discusses our current understanding of molecular mechanisms underpinning fraction size sensitivity and highlights its relevance in the context of immune oncology.

Cellular Proliferation and Fraction Size Sensitivity

Normal tissue responses to radiotherapy provide us with a clear inverse association between proliferative indices and fraction size sensitivity [5]. For example, gastrointestinal mucosa and epidermis have relatively high proliferative indices and are insensitive to fraction size, whereas late-reacting normal tissues, such as kidney and spinal cord, have low proliferative indices and are very sensitive to fraction size [6]. The hypothesis that the same association extends to tumours has been tested in translational studies using diagnostic tissue from the START and CHHiP randomised trials of fractionation in breast and prostate cancer, respectively [7,8]. The START trials included START-P (pilot), START-A and START-B, which collectively recruited 5861 women with early breast cancer. In START-P and START-A, a regimen of 50 Gy in 25 fractions over 5 weeks was compared with 42.9 Gy, 41.6 Gy or 39 Gy in 13 fractions over 5 weeks (maintaining the same overall treatment time). In the pragmatic START-B, a regimen of 50 Gy in 25 fractions over 5 weeks was compared with 40 Gy in 15 fractions over 3 weeks. For the CHHiP trial, 3216 men with localised prostate cancer were randomised 1:1:1 to receive a standard fractionation schedule of 74 Gy in 37 fractions or one of two hypofractionated schedules: 60 Gy in 20 fractions or 57 Gy in 19 fractions.

In both of the above translational studies, proliferation was assessed using immunohistochemistry for Ki67. Primary breast cancer resection specimens from 181 evaluable patients in the START-P and -A trials who had experienced local recurrence were evaluated [9]. Using diagnostic biopsies from patients in CHHiP, 173 cases with recurrence were matched to 173 controls without recurrence [10]. Both studies found no association between proliferation and recurrence according to fractionation schedule, although in Trans-CHHiP Ki67 did predict recurrence independently of established prognostic factors, including Gleason score [10].

Although both these studies provide reassurance that modestly hypofractionated schedules do not lead to inferior outcomes for breast and prostate tumours with high proliferative indices, they do not definitively disprove a link between proliferation and fraction sensitivity. Bearing in mind that the difference in fraction sizes is modest in both trials, these studies suggest that proliferation index alone is insufficient to discriminate between tumour fraction size sensitivities. In CHHiP, the hypofractionated schedules had a shorter overall treatment time than standard fractionation, leading to a potential confounding effect due to accelerated repopulation. Finally, most patients in CHHiP received androgen deprivation therapy, which exerts an

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https://doi.org/10.1016/j.clon.2018.07.001

antiproliferative effect [11], possibly weakening the association with fraction size sensitivity [8].

DNA Repair and Fraction Size Sensitivity

At the molecular level, proliferating versus non-proliferating cells process their radiation-induced DNA double-strand breaks differently. G0/G1 cells rely heavily on error-prone non-homologous end joining (NHEJ) and alternative NHEJ, whereas S/G2 cells are able to use high fidelity homologous recombination [12]. Defective NHEJ has been associated with loss of fraction sensitivity and homologous recombination can mediate resistance to fraction size sensitivity [13,14]. Pre-clinical studies deciphering this mechanism have been previously described [15]. Elucidating the functionality of NHEJ versus homologous recombination using diagnostic tumour tissue where a genotoxic treatment has not yet been delivered is challenging. Proficiency of homologous recombination has been successfully evaluated using pre- and post-chemotherapy evaluation of RAD51 foci [16,17]. Delivery of 'test dose' radiotherapy is not currently feasible before deciding optimal fractionation, although *ex vivo* irradiation has been tested [18,19]. Next generation sequencing of DNA repair genes is increasingly used for the selection of targeted therapy in castration-resistant prostate cancer [20]. There may, therefore, be a future role for targeted sequencing of DNA repair genes to assist individualised radiotherapy fractionation.

P53 and Fraction Size Sensitivity

Our group has recently shown that fraction size sensitivity, measured by split-dose recovery in a range of normal and malignant human cells, is dependent on the presence of wild-type p53 [21]. Prostate tumour cells with mutant p53 (PC3) showed no difference in survival when irradiated with 4 × 1 Gy daily fractions versus 4 Gy acute dose in contrast to p53 wild-type cells (LNCaP) [21]. p53 mutation is a relatively uncommon event in primary prostate cancer (occurring in 8% of tumours [4]) and is consistent with the above pre-clinical observations that prostate tumours on average show a high fraction size sensitivity [8,22]. By contrast, p53 mutation in lung cancer is much more common (81% of squamous cell tumours) [1,2]. Lung tumours tend to show much less fraction sensitivity and have a much higher average alpha/beta ratio than prostate tumours [23]. A study measuring tumour growth delay in two genetic variants of a lung adenocarcinoma mouse model after either a single fraction of 11.6 Gy or two fractions of 7.3 Gy found no statistically significant difference in the response of lung tumours deficient in p53 to the single versus two smaller doses in contrast to tumours with wild-type p53 [24]. If pre-clinical observations hold true in human tumours, it may be possible to improve radiotherapy response by using hypofractionated schedules in p53 wild-type tumours and standard fractionation to a higher total dose in p53 mutant tumours.

Fraction Size in the Context of the Immune Response to Radiotherapy

In the era of immune oncology, an improved understanding of how radiation-induced cell kill contributes to the immune response and vice versa, including the impact of different fractionation schedules, is a research priority. The synergy of radiation with immune checkpoint blockade (ICB) is a particular research focus at present [25]; however, the immune response according to fractionation is also likely to be important when using radiotherapy alone in the curative setting.

The specific radiotherapy fraction size used appears to be important in achieving the so-called abscopal response to radiation plus ICB. Using TSA mouse mammary carcinomas and MCA38 mouse colorectal carcinomas in syngeneic immunocompetent C57BL/6 mice, synergy with CTLA4 blockade in terms of distant control was better using 3 × 8 Gy than a single 20 Gy fraction [26]. Fractions of 8 Gy enabled maximal induction of cytosolic DNA and a subsequent type 1 interferon response via cGAS/STING. However, with 20 Gy the DNA exonuclease Trex1 was induced, which degraded cytosolic DNA, thus precluding downstream production of interferon beta.

Translation of these mechanistic insights to human cancers offers potential to maximise the abscopal response using ICB/radiation combinations for metastatic disease, and may also improve control of micro-metastases in locally advanced disease. The total dose needs to be considered alongside fraction size, as this also impacts synergy with ICB [26]. Further challenges in a clinical context include integrating the above with chromosomal instability, which varies between tumours [27]. Micronuclei arising spontaneously from chromosomal instability can spill genomic DNA into the cytoplasm, which, via cGAS/STING, activates downstream non-canonical NF-KB signalling, rather than type 1 interferons [28].

Data assessing the impact of fractionation on other aspects of the innate immune response are currently lacking. Low dose irradiation (2 Gy) was shown to promote infiltration of anti-tumour inducible nitric oxide synthase (iNOS) expressing macrophages, which were important for subsequent T cell recruitment and vascular normalisation; however, higher fraction sizes and doses were not evaluated in this study [29].

The impact of fractionation on the adaptive immune response is also likely to be clinically important. Neoantigen burden is an important predictive factor for the response to ICB [30]. It has been proposed that radiotherapy may increase sub-clonal neoantigens, potentially causing T cell exhaustion [31], although to our knowledge this has not been shown in patients receiving radiotherapy. In a pre-clinical context, five daily fractions of 2 Gy lead to polyclonal expansion of TCR clones in irradiated CT26 murine colon tumours, which were predominantly those that existed before radiotherapy, rather than new clones [32]. Treatment with other fractionation schedules of 3 × 12 Gy or a single dose of 7 Gy gave similar findings.

Please cite this article in press as: Wilkins A, et al., Science in Focus: Biological Optimisation of Radiotherapy Fraction Size in an Era of Immune Oncology, Clinical Oncology (2018), https://doi.org/10.1016/j.clon.2018.07.001