Science in Focus; Biological optimisation of radiotherapy fraction size in an era of immune-oncology

Anna Wilkins[1,2], Alan Melcher[1,2], Navita Somaiah[1,2]

1. Division of Radiotherapy and Imaging, The Institute of Cancer Research, London
2. Royal Marsden Hospital, London

Corresponding author: Navita Somaiah, Royal Marsden Hospital, Downs Road, Sutton SM2 5PT. Email: navita.somaiah@icr.ac.uk. Tel: 020 8661 3371

Key words: radiotherapy fractionation, fraction size sensitivity, immune response, radio-immunotherapy

Conflict of interest: The authors declare no conflict of interest

Acknowledgements: We acknowledge NHS funding to the National Institute for Health Research (NIHR) Biomedical Research Centre at the Royal Marsden NHS Foundation Trust and the Institute of Cancer Research

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 sizeunderpinning the linear-quadratic model. This article will discuss our current understanding of molecular mechanisms underpinning fraction size sensitivity, and highlight its relevance in the context of immuneoncology.

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 anti-proliferative 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 (DSB) 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 (HR) [12]. Defective NHEJ has been associated with loss of fraction sensitivity and HR can mediate resistance to fraction size sensitivity [13, 14]. Pre-clinical studies deciphering this mechanism have been previously described in this journal [15]. Elucidating the functionality of NHEJ versus HR using diagnostic tumour tissue where a genotoxic treatment has not yet been delivered is challenging. Proficiency of HR has been successfully evaluated using pre and post chemotherapy evaluation of RAD51 foci [16, 17]. Delivery of "test dose" radiotherapy is not currently feasible prior to deciding optimal fractionation, although ex-vivo irradiation has been tested [18, 19]. Next-generation sequencing of DNA repair genes is increasingly used for 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 (WT) p53 [21]. Prostate tumour cells with mutant p53 (PC3) showed no difference in survival when irradiated with 4x1Gy daily fractions versus 4Gy acute dose in contrast to p53 WT 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]. In 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

11.6Gy or two fractions of 7.3Gy, 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 WT 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 WT 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 3x8Gy than a single 20Gy fraction [26]. Fractions of 8Gy enabled maximal induction of cytosolic DNA and a subsequent type 1 interferon response via cGAS/STING. However, with 20Gy 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. 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 is currently lacking. Low dose irradiation (2Gy) was shown to promote infiltration of anti-tumour 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. Neo-antigen burden is an important predictive factor for 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 demonstrated in patients receiving radiotherapy. In a pre-clinical context, 5 daily fractions of 2Gy lead to polyclonal expansion of TCR clones in irradiated CT26 murine colon tumours, which were predominantly those that existed prior to radiotherapy, rather than new clones [32]. Treatment with other fractionation schedules of 3x12Gy or a single dose of 7Gy gave similar findings.

A different study explored the impact of different fractionation schedules on the tumour microenvironment using the CT26 and MCA38 tumour models [33]. A single ablative dose of radiation (30Gy) transformed the immunosuppressive tumour microenvironment resulting in an intense CD8+ T cell tumour infiltrate, and a loss of myeloid derived suppressor cells (MDSCs). Much higher rates of tumour control occurred than with lower single doses of 15Gy and 20Gy, or 10x3Gy of radiation. Interestingly, addition of 10x3Gy after 1x30Gy markedly reduced tumour control compared to a single fraction of 30Gy. The CD8 T cell infiltrate reduced at day 35 from approximately 70% with the single dose alone, to 4-8% with fractionated

radiation alone or in combination with the single high dose. This finding could have important implications for repeated irradiation in the clinic.

A further study compared 15Gy, 5x3Gy and no irradiation in B16/OVA murine melanoma tumours [34]. The authors systematically compared antigen presentation in tumour draining lymph nodes, priming and expansion of tumour-reactive T cells, tumour immune cell infiltration into the tumour, T cell effector function and tumour cell kill between fractionation schedules. All aspects of the immune response mentioned above were highest using the single 15Gy schedule. The enhanced trafficking and infiltration of tumour-specific T cells with 1x15Gy versus 3x3Gy may once again be due to repeated radiation treatments killing activated T cells.

Finally, using entirely different tumour models of T and B cell lymphoma, Dovedi et al showed that the Toll-like receptor (TLR7) agonist, R848 in combination with RT led to long-lasting clearance of tumour [35]. Intriguingly, in this model, fractionated radiotherapy (5x2Gy) enhanced efficacy of this combination versus a single 10Gy fraction. Induction of tumour-specific memory was demonstrated with both fractionation schedules.

The above pre-clinical findings suggest that the immune response to different radiotherapy fraction sizes depends on the murine model used and on the ICB, if relevant. There has been a rapid expansion in clinical trials using radiotherapy/immunotherapy combinations which use a wide range of dose/fractionation schedules [25]. Unfortunately, choice of fraction size is not underpinned by strong pre-clinical or clinical data and we do not currently have a good understanding of the mechanistic basis of differences in immune response according to fraction size. Furthermore, research to date has focussed largely on the abscopal response; the immunological effects of different fractionation schedules on local tumour control in the curative setting also need consideration. Answering such questions is a research priority which is important to address using a wide range of pre-clinical models and well-designed human studies with translational endpoints.

Our understanding of the association of the DNA damage response with the immune response continues to grow and exciting potential remains for biological tailoring of radiotherapy fractionation according to cell cycle checkpoints, DNA repair and the immune response.

References

[1] Cancer Genome Atlas Research N. Comprehensive genomic characterization of squamous cell lung cancers. Nature. 2012;489:519-25.

[2] Cancer Genome Atlas Research N. Comprehensive molecular profiling of lung adenocarcinoma. Nature. 2014;511:543-50.

[3] Cancer Genome Atlas N. Comprehensive molecular portraits of human breast tumours. Nature. 2012;490:61-70.

[4] Cancer Genome Atlas Research N. The Molecular Taxonomy of Primary Prostate Cancer. Cell. 2015;163:1011-25.

[5] Thames HD, Jr., Withers HR, Peters LJ, Fletcher GH. Changes in early and late radiation responses with altered dose fractionation: implications for dose-survival relationships. Int J Radiat Oncol Biol Phys. 1982;8:219-26.

[6] Stewart FAaVdK, A. Proliferative and cellular organization of normal tissues. Basic Clinical Radiobiology, GG Steel, Editor 2002, Arnold: London.23-9.

[7] Haviland JS, Owen JR, Dewar JA, Agrawal RK, Barrett J, Barrett-Lee PJ, et al. The UK Standardisation of Breast Radiotherapy (START) trials of radiotherapy hypofractionation for treatment of early breast cancer: 10-year follow-up results of two randomised controlled trials. Lancet Oncol. 2013;14:1086-94.

[8] Dearnaley D, Syndikus I, Mossop H, Khoo V, Birtle A, Bloomfield D, et al. Conventional versus hypofractionated high-dose intensity-modulated radiotherapy for prostate cancer: 5-year outcomes of the randomised, non-inferiority, phase 3 CHHiP trial. The Lancet Oncology. 2016;17:1047-60.

[9] Somaiah N, Rodrigues D, Cheang M, Heindl A, Daley F, Boyle S, et al. Tumour characteristics associated with local relapse after hypofractionated radiotherapy in early breast cancer (Abstract OC-0392, Supplement 1, 3rd ESTRO Forum). Radiother Oncol. 2015;115.

[10] Wilkins AC GB, Szijgyarto Z, Haviland J, Griffin C, Stuttle C, Daley F, Corbishley CM, Dearnaley DP, Hall E, Somaiah N; on behalf of the CHHiP Trial Investigators.

Ki67 Is an Independent Predictor of Recurrence in the Largest Randomized Trial of 3 Radiation Fractionation Schedules in Localized Prostate Cancer. International journal of radiation oncology, biology, physics. 2018.
[11] Tarish FL, Schultz N, Tanoglidi A, Hamberg H, Letocha H, Karaszi K, et al. Castration radiosensitizes prostate cancer tissue by impairing DNA double-strand break repair. Science translational medicine. 2015;7:312re11.
[12] Ceccaldi R, Rondinelli B, D'Andrea AD. Repair Pathway Choices and Consequences at the Double-Strand Break. Trends in cell biology. 2015.
[13] Somaiah N, Yarnold J, Daley F, Pearson A, Gothard L, Rothkamm K, et al. The relationship between homologous recombination repair and the sensitivity of human epidermis to the size of daily doses over a 5-week course of breast radiotherapy. Clinical cancer research : an official journal of the American Association for Cancer Research. 2012;18:5479-88.
[14] Somaiah N, Yarnold J, Lagerqvist A, Rothkamm K, Helleday T. Homologous recombination mediates cellular resistance and fraction size sensitivity to radiation therapy. Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology. 2013;108:155-61.
[15] Somaiah N, Rothkamm K, Yarnold J. Where Do We Look for Markers of Radiotherapy Fraction Size Sensitivity? Clin Oncol (R Coll Radiol). 2015;27:570-8.
[16] Graeser M, McCarthy A, Lord CJ, Savage K, Hills M, Salter J, et al. A marker of homologous recombination predicts pathologic complete response to neoadjuvant chemotherapy in primary breast cancer. Clin Cancer Res. 2010;16:6159-68.
[17] Bhide SA, Thway K, Lee J, Wong K, Clarke P, Newbold KL, et al. Delayed DNA double-strand break repair following platin-based chemotherapy predicts treatment response in head and neck squamous cell carcinoma. British journal of cancer. 2016;115:825-30.
[18] De-Colle C, Yaromina A, Hennenlotter J, Thames H, Mueller AC, Neumann T, et al. Ex vivo gammaH2AX radiation sensitivity assay in prostate cancer: Inter-patient

and intra-patient heterogeneity. Radiother Oncol. 2017;124:386-94.
[19] Willers H, Taghian AG, Luo CM, Treszezamsky A, Sgroi DC, Powell SN. Utility of DNA repair protein foci for the detection of putative BRCA1 pathway defects in breast cancer biopsies. Mol Cancer Res. 2009;7:1304-9.
[20] Mateo J, Carreira S, Sandhu S, Miranda S, Mossop H, Perez-Lopez R, et al. DNA-Repair Defects and Olaparib in Metastatic Prostate Cancer. N Engl J Med. 2015;373:1697-708.
[21] Somaiah N, Anbalagan S, Strom C, Downs J, Jeggo P, Wilkins A, et al. Cell cycle checkpoint modulates radiotherapy fraction size sensitivity in normal and malignant cells (abstract OC-0380). Radiother Oncol. 2018;127.
[22] Brenner DJ, Hall EJ. Fractionation and protraction for radiotherapy of prostate carcinoma. Int J Radiat Oncol Biol Phys. 1999;43:1095-101.
[23] Thames HD, Bentzen SM, Turesson I, Overgaard M, van den Bogaert W. Fractionation parameters for human tissues and tumors. International journal of radiation biology. 1989;56:701-10.
[24] Perez BA, Ghafoori AP, Lee CL, Johnston SM, Li Y, Moroshek JG, et al. Assessing the radiation response of lung cancer with different gene mutations using genetically engineered mice. Frontiers in oncology. 2013;3:72.
[25] Kang J, Demaria S, Formenti S. Current clinical trials testing the combination of immunotherapy with radiotherapy. J Immunother Cancer. 2016;4:51.
[26] Vanpouille-Box C, Alard A, Aryankalayil MJ, Sarfraz Y, Diamond JM, Schneider RJ, et al. DNA exonuclease Trex1 regulates radiotherapy-induced tumour immunogenicity. Nat Commun. 2017;8:15618.
[27] Sansreget L VB, Swanton C. Determinants and clinical implications of chromosomal instability in cancer. Nature Reviews Clinical Oncology. 2018;15:139-50.
[28] Bakhoum SF, Ngo B, Laughney AM, Cavallo JA, Murphy CJ, Ly P, et al. Chromosomal instability drives metastasis through a cytosolic DNA response.

Nature. 2018;553:467-72.
[29] Klug F, Prakash H, Huber PE, Seibel T, Bender N, Halama N, et al. Low-dose irradiation programs macrophage differentiation to an iNOS(+)/M1 phenotype that orchestrates effective T cell immunotherapy. Cancer Cell. 2013;24:589-602.
[30] Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science. 2015;348:124-8.
[31] McGranahan N, Furness AJ, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science. 2016;351:1463-9.
[32] Dovedi SJ, Cheadle EJ, Popple AL, Poon E, Morrow M, Stewart R, et al. Fractionated Radiation Therapy Stimulates Antitumor Immunity Mediated by Both Resident and Infiltrating Polyclonal T-cell Populations when Combined with PD-1 Blockade. Clinical cancer research : an official journal of the American Association for Cancer Research. 2017;23:5514-26.
[33] Filatenkov A, Baker J, Mueller AM, Kenkel J, Ahn GO, Dutt S, et al. Ablative Tumor Radiation Can Change the Tumor Immune Cell Microenvironment to Induce Durable Complete Remissions. Clinical cancer research : an official journal of the American Association for Cancer Research. 2015;21:3727-39.
[34] Lugade AA, Moran JP, Gerber SA, Rose RC, Frelinger JG, Lord EM. Local radiation therapy of B16 melanoma tumors increases the generation of tumor antigen-specific effector cells that traffic to the tumor. Journal of immunology. 2005;174:7516-23.
[35] Dovedi SJ, Melis MH, Wilkinson RW, Adlard AL, Stratford IJ, Honeychurch J, et al. Systemic delivery of a TLR7 agonist in combination with radiation primes durable antitumor immune responses in mouse models of lymphoma. Blood. 2013;121:251-9.