Biology news in a FLASH

The last update on research into the use of ultra-high-dose-rate (UHDR, known as FLASH) radiotherapy was offered through this channel in June 2022, so it is high time to zoom in on what is happening in this very busy research field, in which review papers are published almost as frequently as are original research articles.
For newcomers to the field, here is an ultra-fast introduction. FLASH radiotherapy is an experimental radiation modality that has been drawing attention within the radiotherapy research community since Vincent Favaudon and colleagues demonstrated in 2014 that use of FLASH radiation led to increased sparing of normal tissue, while the tumour response was unaltered, compared with radiotherapy applied at usual dose rates [1]. The following is my very personal selection and comments about what has emerged recently on the FLASH data front.

Fractionation
Since the vast majority of pre-clinical FLASH studies have been performed with high doses delivered in a single fraction, one of the more urgent questions within FLASH research is whether or not the FLASH effect is maintained if doses are fractionated. One paper published in 2021 suggested that with certain fractionation schemes, it was possible to maintain a FLASH effect, but with others it was not [2]. This study involved consideration of memory skills and tumour growth delay in glioblastoma-bearing mice as endpoints. Two papers have been published since then, including some by the same authors, in which the impact of fractionated FLASH treatment on whole-brain-irradiated mice was investigated in terms of neurological endpoints. The radiation effect on long-term potentiation after delivery of 2x10Gy [3] to juvenile mice or 10x3Gy [4] to adult mice was investigated. It was demonstrated for both treatments that the response in the FLASH-irradiated mice resembled that in the control mice. When looking at the control data in the two studies, it is apparent that the level of the response is not dose-dependent but rather that there must be a dose threshold, which is also noted by the authors, and that at the given doses at FLASH dose rates, this threshold is not reached. These fractionation data are good news from the perspective of clinical implementation of FLASH. However, a lot of open questions remain regarding fractionation and FLASH, including how to establish the FLASH effect with fractionation in a system in which the FLASH effect can be quantified.

Tumour response
The greatest interest in FLASH studies has been focused on the sparing effect in normal tissues and which factors influence this response. However, the tumour response is a crucial factor. If FLASH is to prove useful to broaden the therapeutic window, it must be shown that the tissue-sparing effect does not occur in tumours, or at least not to the same extent as is seen in normal tissue.
In a systematic review of studies of the FLASH effect in preclinical tumour models, 66 datasets were identified from 15 publications in which UHDR and conventional doses (CONV) had been compared in terms of anti-tumour efficacy. In the studies, the tumour response to FLASH irradiation was investigated predominantly through the use of growth assays; only a limited number of studies had focused on tumour control as endpoint, and in only one of the studies had the tumour-controlling dose (TCD50) been determined [5]. The studies reported predominantly non-significant response differences; in 12 of the 15 studies, isoefficacy was demonstrated for FLASH and CONV irradiation of tumours. This is promising for the differential effect, but as explained by the authors, biological

variations among tumours could affect their responses, and more studies of the control of tumours that represent different histological and radiobiological factors are now needed.

FLASH Mechanism

The underlying mechanism behind the differential response between normal tissues and tumours remains to be clarified, and one of the big questions within FLASH research is “what is going on?” Although several mechanisms for the FLASH effect have been proposed, including radiolytic oxygen depletion and differential effects on inflammatory and immune responses as well as on the vasculature, so far none of them has been adequately validated. A recent, thorough review has gone through the different hypotheses and the supporting experimental data [6]. One of the most debated hypotheses is the oxygen depletion theory, which argues that more oxygen is consumed at UHDRs because considerably more electrons are liberated per unit of time than at CONV dose rates, and that this leads to a transient state of hypoxia that induces radioprotection. More experimental data have supported the hypothesis that oxygen plays a role in mediating the FLASH effect, but it has been argued, mostly based on modelling studies, that the consumption of oxygen is too low to have a biological impact that on its own could explain the FLASH effect, as complete depletion of oxygen seems unlikely to occur after UHDR irradiation. A recent study involved the measurement of intracellular levels of oxygen during the administration of FLASH radiation in vitro. This demonstrated that the differences in changes of oxygen levels at FLASH and CONV dose rates, which had been detected previously in solution-based experiments, disappeared when measurements were conducted inside cells. The researchers concluded that depletion of oxygen in in-vitro cells that was caused by a clinical dose of proton radiation delivered as FLASH did not lead to a transient state of hypoxia [7].
In another study, whole blood was irradiated with 20Gy electrons at various oxygen levels and dose rates, and DNA damage was assessed through use of the comet assay. The results demonstrated that application of FLASH radiotherapy induced lower levels of DNA damage than did application of CONV irradiation. The amount of DNA damage was modulated by the oxygen tension, and increased with the total dose and dose rate of irradiation. This finding supports the hypothesis that an oxygen-related mechanism, such as the above mentioned oxygen depletion, contributes to the tissue-sparing effect of FLASH irradiation [8]. In order to improve understanding of the mechanism behind the FLASH effect, the same group continued to use the comet assay to profile the FLASH-induced damage, in order to distinguish between the mechanisms involved in the differential level of DNA damage. This did not demonstrate any evidence of any crosslink formation, which would support the idea of radical-radical recombination. Instead it demonstrated a more anoxic profile of induced damage, which supported transient oxygen depletion as the mechanism behind the FLASH effect [9].
It is an exciting time for us FLASHisists. The question of whether or not you are a FLASH believer is not now so much about whether there is a FLASH effect, as this is hard to contradict. Rather it is about whether you believe that FLASH can make a broad-scale clinical entrance and be the big game-changer that has been discussed. To decide either for or against that, we still have a lot of experimental work to do, and the data that come in over the next few years will most likely decide that point.

Brita Singers Sørensen,
Aarhus University, Denmark, and Erasmus Medical Center, The Netherlands

[1] Favaudon V, Caplier L, Monceau V, Pouzoulet F, Sayarath M, Fouillade C, et al. Ultrahigh dose-rate FLASH irradiation increases the differential response between normal and tumor tissue in mice. Sci Transl Med 2014;6:245ra93.
[2] Montay-Gruel P, Acharya MM, Jorge PG, Petit B, Petridis IG, Fuchs P, et al. Hypofractionated FLASH-RT as an effective treatment against glioblastoma that reduces neurocognitive side effects in mice. Clin Cancer Res 2021;27:775–84.
[3] Allen BD, Alaghband Y, Kramár EA, Ru N, Petit B, Grilj V, et al. Neuro-Oncology effect in juvenile mice exposed to hypofractionated 2022;25:927–39.
[4] Limoli CL, Kramár EA, Almeida A, Petit B, Grilj V, Baulch JE, et al. The sparing effect of FLASH-RT on synaptic plasticity is maintained in mice with standard fractionation. Radiother Oncol 2023;186:109767.
[5] Böhlen TT, Germond J-F, Petersson K, Ozsahin EM, Herrera FG, Bailat C, et al. Effect of Conventional and Ultrahigh Dose Rate FLASH Irradiations on Preclinical Tumor Models: A Systematic Analysis. Int J Radiat Oncol Biol Phys 2023;117:1007–17.
[6] Bogaerts E, Macaeva E, Isebaert S, Haustermans K. Potential Molecular Mechanisms behind the Ultra-High Dose Rate "FLASH" Effect. Int J Mol Sci 2022;23.
[7] Khatib M El, Motlagh AO, Beyer JN, Troxler T, Allu SR, Sun Q, et al. Direct Measurements of FLASH-Induced Changes in Intracellular Oxygenation. Int J Radiat Oncol 2023.
[8] Cooper CR, Jones D, Jones GD, Petersson K. FLASH irradiation induces lower levels of DNA damage ex vivo, an effect modulated by oxygen tension, dose, and dose rate. Br J Radiol 2022;95.
[9] Cooper CR, Jones DJL, Jones GDD, Petersson K. Comet Assay Profiling of FLASH-Induced Damage: Mechanistic Insights into the Effects of FLASH Irradiation. Int J Mol Sci 2023;24:1–15.