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History and current perspectives on the biological effects of high-dose spatial fractionation and high dose-rate approaches: GRID, Microbeam & FLASH radiotherapy

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Abstract

The effects of various forms of ionizing radiation are known to be mediated by interactions with cellular and molecular targets in irradiated and in some cases non-targeted tissue volumes. Despite major advances in advanced conformal delivery techniques, the probability of normal tissue complication (NTCP) remains the major dose-limiting factor in escalating total dose delivered during treatment. Potential strategies that have shown promise as novel delivery methods in achieving effective tumour control whilst sparing organs at risk involve the modulation of critical dose delivery parameters. This has led to the development of techniques using high dose spatial fractionation (GRID) and ultra-high dose rate (FLASH) which have translated to the clinic. The current review discusses the historical development and biological basis of GRID, microbeam and FLASH radiotherapy as advanced delivery modalities that have major potential for widespread implementation in the clinic in future years.

Introduction

Radiotherapy remains the most effective non-surgical cancer treatment modality (1). The speciality has undergone an unparalleled period of development over the past two decades due to major technical advances in treatment planning, delivery and understanding of radiobiological response at the cell, tissue and whole organism levels (2-6). This has enabled the implementation of advanced delivery modalities into routine clinical practice including intensity-modulated radiotherapy (IMRT) and volumetric modulated arc therapy (VMAT). However, these approaches remain limited by dose constraints in surrounding organs at risk (OARs). This has led to the development of novel delivery modalities that aim to exploit physical beam delivery parameters for biologically optimised treatments.

The goal of this review of spatial fractionation and ultra-high dose rate radiotherapy for the 125th anniversary issue of the *BJR* is to refresh our current perspectives on the potential of increasing therapeutic efficacy through innovative local radiation exposures, and the possible underlying biological mechanisms that can be exploited for patient benefit.

Spatially fractionated radiotherapy (GRID): Spatially fractionated radiotherapy challenges the conventional paradigm of radiotherapy by aiming to deliver a highly heterogeneous dose to the tumour volume. The concept of GRID was first demonstrated over a century ago by Alban Köhler (7) and was developed as a means of reducing skin injury with the low energy X-rays by placing an attenuating grid directly on the skin of the patient to reduce skin dose directly under the grid. At a depth of several millimetres, the uniform dose distribution is lost and so does not affect the dose distribution in the tumour (8). Evidence from both clinical and preclinical studies has clearly demonstrated that dividing the target volume into discrete sub-volumes can limit normal tissue damage from orthovoltage sources which have been recently been reviewed (9, 10). The possible biological mechanisms and extent to which GRID

1 contributes to overall tumour control in the context of modern-day chemoradiation regimens is
2 not clearly understood.
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4 Interestingly, several *in vitro* and *in vivo* studies have implicated radiation-induced
5 abscopal effects as potential mediators of response to GRID (11-14). The first introduction to
6 the concept of abscopal effects was made in a report published by the *British Journal of*
7 *Radiology* for the first time suggesting that radiation responses may occur remotely from local
8 or whole body irradiation scenarios (15, 16). In the later half-century and beyond, there have
9 been a growing numbers of reports describing observations of the abscopal effect
10 experimentally and in the clinic (17-25). The prospect that GRID can increase total tumour
11 dose and possibly activate beneficial bystander or abscopal immune effects while minimizing
12 effects to normal tissues offers promising options for therapy.
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26 Whilst the abscopal effect remains a controversial and poorly controlled phenomenon,
27 accurate assessment of tumour and immune response through circulating biomarkers may
28 enable more widespread integration of GRID in the clinic. As advanced imaging and
29 radiotherapy technologies continue to develop, the ability to deliver GRID has greatly
30 improved. Whether or not the advanced technology and treatment planning available for new
31 beam geometries can finally unlock the full potential of abscopal effects remains an open
32 question. Ultimately, it may require integrating knowledge of complex radiobiological
33 mechanisms driving these responses with innovative technological solutions.
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Ultra-High dose rate applications (FLASH and microbeam): In addition to optimised
spatial delivery of radiation beams, there is currently major interest in exploiting unique
radiobiological responses occurring at mean dose rates in excess >40 Gy/s, delivered as a single
or a few pulses over intervals of milliseconds, known as FLASH-RT. It is important to note
that ultra-high dose rates have been used for decades in spatial fractionation applications known

as microbeam radiation therapy (MRT). However, recent work and technological advances in radiation delivery and control have allowed open field FLASH research which has resulting in the successful treatment of a patient with multi-resistant T-cell cutaneous lymphoma (26). FLASH-RT has the potential to deliver a paradigm shift in curative radiation therapy, yet the mechanistic underpinnings of response remain largely unknown but are thought to be due radiochemical depletion of oxygen at ultra-high dose rates which affects the radioresistance conferred on the irradiated normal tissue more than on tumor tissue (27-30).

On a basic level both FLASH and GRID represent alternative dose delivery modalities that can exploit certain differences between normal tissue and tumours. These techniques have resulted in unexpected beneficial effects that could not be predicted based on classical understanding of dose rate effect and targeted responses. It should be noted that while the potential of each seems large, there is not an expected sparing from late tissue reactions in the irradiated volumes since the fraction sizes are quite large. Understanding the biological underpinnings and the optimum physical parameters of these will require significant multidisciplinary effort to drive further biologically-based clinical trials using these techniques (29).

Classifications and delivery of major forms of SFRT

Spatially fractionated conventional radiotherapy (GRID): The field of GRID is the closest manifestation of the early work by Kohler and many others using relatively low energy photons and a screen or patterned blocking sheet to divide the exposure into high and low exposure segments. As the development of higher voltage sources occurred, there began to be interest in re-evaluating the original GRID concept to avoid normal tissue morbidities at depth. Mohuiddin and his associates were the pioneers of these studies beginning in the 1980's and continuing until today (31-33). A number of other centres have also joined the approach in the

hope of providing their patients with a boost of anti-tumour control while keeping normal tissue responses at an acceptable level. For example, Peñagaricano has demonstrated this nicely in a small series of patients (34). The approach was initially implemented in 3D plans with one or two fields created by MLC shaping, cerrobend blocks or customized blocks that can be placed in the linear accelerator head (35, 36). MLC or cerrobend based GRID makes the implementation straightforward. This has the potential benefit of implementing GRID in developing countries in which financial constraints limit the use of advanced GRID techniques. After initial studies over the years suggested that patients may benefit from GRID applications to their tumour, several new strategies for applying GRID with photons in IMRT treatment plans or with protons have emerged with advancing technological capabilities (36-38). These advanced GRID techniques allow for the delivery of spatial fractionation to deep seated bulky tumours and dose sparing to normal tissues. However, it remains to be seen if the dose distribution is sharp enough to retain optimal/effective peak to valley ratios related to the biological mechanisms of action (Figure 1). Nonetheless, these additional options expand the possibilities of utilizing spatial fractionation in a wide variety of patients, with some indications that the therapeutic benefit remains in deep seated tumours (39). Even after 100+ years of clinical and research experience, the use of GRID in the clinic remains a simple strategy to avoid normal tissue damage while increasing the dose to the tumour (Figure 2). Advanced mechanisms of action and potential synergism with cutting-edge drug or antibody therapies are still to be fully exploited, which is a current inspiration of a number of centres around the world.

Microbeam (MRT with photons or protons): Very high dose rates of the order of up to 100 Gy/s or higher have been investigated for decades in a spatial fraction format using synchrotron generation and known as microbeam therapy (40, 41). There is much to learn from the early work done by a number of groups that employed the synchrotron-produced beams at

1 Brookhaven National Laboratory in New York, and the European Synchrotron Research
2 Facility in Grenoble, France among others (42-44). One of the original questions was, what
3 width of beam in a repeating pattern could be used without increasing normal tissue damage.
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5 It was found that beam widths approaching 0.5 mm or higher became quite tissue destructive,
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7 especially when repeated at centre to centre distances of the same width, while below that there
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9 was relative tissue sparing, even when the peak doses were in the 100s of Gy. Part of this
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11 result may have to do with the unique way in which normal cells vs. tumour cells react to ultra-
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13 high dose rates, and part of the explanation lies in the preservation of tissue stem cell/functional
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15 units at a level and distribution that allows tissue repair and function to resume quickly after
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17 exposure.
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26 **Ultra-high dose rate radiotherapy (FLASH with electrons, photons or protons):** The
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28 concept of ultra-high dose applications in radiotherapy has been experiencing an explosion of
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30 interest from all three of the major disciplines that make up the field of radiotherapy: clinical,
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32 biology and physics. The interest is for good reason, since a number of recent publications have
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34 demonstrated an enhanced therapeutic ratio when ultra-high dose rate (typically characterized
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36 by 40 Gy/s or greater) are applied to model tumour systems in several animal models (29, 45-
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38 47). As well as dose-rate, it is clear that other parameters such as the intra-pulse dose rate,
39
40 other beam parameters such as frequency (Hz), overall time of exposure, oxygenation and the
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42 target volume are also important (27, 48). The majority of the FLASH experiments and clinical
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44 applications in certain isolated cases have been with electron beams which necessitate a
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46 superficial target and can be applied to large areas, if not volumes. However, there are
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48 demonstrations in the literature that with both photons and protons, or other heavy ions, flash
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50 is possible and some biological advantages are also demonstrated using advanced
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52 modifications of existing equipment and heavy ion or proton facilities (49-52). Although there
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are distinct differences in the concept of spatial and FLASH radiotherapy applications, high doses and dose-rates are a constant requirement and the variations of biological responses to each beam size and dose rate combination will need to be carefully studied for optimal applications (Figure 3).

Biological mechanisms of response to demonstrated and theorized

Bystander effects: One of the phenomena surrounding spatial distribution of radiation exposure has long been thought to be a type of bystander effect. Original bystander work was done mostly in vitro and mostly with low dose exposures (53-56). Their clinical impact was quickly realised (57). and a number of groups have now demonstrated bystander effects, including cell killing, when high dose spatial fractionation is applied either in vitro or in vivo (14, 58, 59). Furthermore, there is evidence of adaptive/bystander like responses within and between modulated fields, which would also have special relevance to the application of GRID or Lattice therapy approaches (60, 61).

Vascular/angiogenic effects: The use of stereotactic, high dose radiotherapy has brought the potential impact of vascular damage to the front line in explaining why many tumours are controlled at total doses that would not be mathematically predicted to kill all cells (62, 63). Thus, it follows that vascular damage is likely important in both conventional X-ray spatial fractionation as well as extremely high dose, spatially fractionated microbeam applications. There have been a number of studies published with a focus on the vascular perturbations, damage and inflammation induced by spatial fractionation (40, 64-66). In addition, a general reaction to vascular stress or damage is an angiogenic response similar to wound healing demonstrated in a number of preclinical studies of spatially fractionated radiotherapy and with obvious implications for tumour control (67).

Immune effects; abscopal influence of local/spatial exposures: In close relationship to the vascular damage that plays a role in the tissue response to high dose spatial and ultra-high dose rate exposures, the immune reaction and general inflammatory cascade appears to have some unique relevant features. This response may be distinctly different in magnitude and outcomes when the dose is spatially fractionated. Unlike whole tumour, high dose exposures where the amount of induced abscopal effect may be dictated by extensive vascular damage and inflammation across the entire tumour volume (25), GRID and microbeam treatment may allow a more intact physiological response to the radiation fields as the low dose volumes retain viable vasculature and immune cells. In agreement with this notion, a number of studies have found that spatial fractionation induces a more beneficial overall immune reaction than other approaches (11, 12). It follows that the production or expression of an abscopal response against remotely located tumours could be enhanced since the irradiated tumour has retained a greater, more functional, ability to recruit immune cells or allow antigens to interact with the immune system for eventual development of a systemic immune attack of the cancer.

Oxygen availability and radiochemistry considerations for conventional spatial fractionation and ultra-high dose rates: There are some recent advances concerning the intracellular mechanisms by which FLASH type of exposures may exert the observed tumour-cell selective effects. The actual mechanisms and applications of FLASH dose rates are far from clear, although a number of groups have postulated that oxygenation radical dynamics/depletion are responsible for the differences between tumour and normal tissue (68, 69) as well as an important role for differential redox biology in tumours relative to normal tissues (28). There are now reports emerging with different model systems that suggest in certain tissues or cells, the FLASH effect may not always be protective in electron or proton irradiation (70, 71). Whether or not whole tissue exposures to ultrahigh dose rates will

ultimately be of clinical benefit, there are also indications that spatial fractionation promotes the reoxygenation and revascularization of residual tumour or normal tissue (40, 67, 72, 73). These variables are critical to understanding the effects of subsequent treatments. Detailed understanding of the relationship of dose and distribution of radiation exposure to physiological changes in the target tissue will greatly facilitate the use of these advanced approaches as a tool in the repertoire of clinical standards of care for a number of cancer types.

Outstanding Challenges for spatial fractionation and ultra-high dose rate approaches

Some promising results with clinical studies using GRID approaches followed by conventional radiation or chemoradiation have been demonstrated (33, 34, 38, 39, 74) and the initial FLASH clinical experience is very intriguing. The idea of using radiation as a tool as a part of optimizing/sequencing with chemotherapy, targeted agents or immunotherapy continues to gain some credibility and as the field of immunotherapy matures, and it may well be the most influential way in which spatial fractionation can be used for patient benefit. A related topic is the possible sparing of immune cells when spatially fractionating the dose. If a reduction in immune cell killing is part of the mechanism that meaningfully contributes to tumour control with GRID or FLASH it would provide even greater rationale for widespread implementation of these approaches. In addition, a few cytokine biomarkers have been studied in the clinical and preclinical studies (for example, TNF- α and IL-2), but little validated logic for using these for either predicting outcome or validating the dosimetry of spatial fractionation or FLASH has been developed. As is typical for promising preclinical therapy approaches, the use of any of these concepts needs further preclinical and, especially, clinical study results to validate their potential for widespread translation.

Current outlook and expected developments

1 It appears that some combination of the GRID/FLASH concepts form the basis of the most
2 intriguing MRT results. In addition, there is the potential of using not only photon-base
3 approaches but also of exploiting charged particles. The Bragg peak of protons or other ion
4 beams can be used to create much cleaner dose distributions, and appears to have a lot of
5 potential to transition spatial fractionation into 21st century, cutting edge radiation therapy
6 delivery techniques. With the demonstrated potential for marked sparing effects using MRT
7 even at very high single doses, the pragmatic approach may be to combine the best of a number
8 of these clearly separate, yet interrelated, approaches and develop these in the future. With
9 the notable uplift in worldwide studies and equipment in development by a number of groups,
10 the field of radiation oncology should soon have a much greater supply of the detailed
11 knowledge needed to make these new (old) tools and concepts available to patients with
12 difficult to manage tumour types and/or locations.
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32 **Figure legends:**

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35 **Figure 1:** Application of spatially fractionated dose distributions to a deep seated pelvic
36 tumour using an intensity modulated radiotherapy (IMRT) based method. **A, C, D:** GTV iso-
37 dose peak and valley dose distribution in axial, coronal, and sagittal views and **B:** dose profile
38 across the axial view. Note the variable dose-gradient slope in the valley dose regions, as
39 well as the difference in peak dose regions due to anatomic placing of the spatially
40 fractionated dose. These variations and valley doses are characteristic features of clinically
41 delivered spatial fractionation that need to be recognized in interpreting mechanisms and
42 outcomes.
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57 **Figure 2:** Example application of spatially fractionated dose in a patient with bulky mass in
58 the neck from carcinoma of the head and neck using a GRID collimator designed to fit in a
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1 linear accelerator. Panel A: Commercially available GRID block for linear accelerator
2 (Radiation Products Design, Inc.). Panel B: Hand drawing of the GRID field as indicated by
3 light guides from linear accelerator on patient that received GRID radiotherapy for a large neck
4 nodal lesion. Panel C: Expected dose at 3 cm and 10 cm in the gross tumour volume using a 6
5 MV linear accelerator. Note how the peak and valley doses are more consistent across the field
6 when using the collimator in comparison to the IMRT application in Figure 1. Prescription was
7 20Gy in 1 fraction. This patient proceeded to have a full course of adjuvant chemo-radiotherapy
8 (66Gy in 30 fractions) after completing GRID therapy.

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22 **Figure 3:** Schematic representation of dose rate and beam sizes for different clinical and
23 experimental radiotherapy techniques.
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Figure 1

FIGURE 1:

FIGURE 2:

Figure 3

FIGURE 3: