# Understanding High-Dose, Ultra-High Dose-Rate and Spatially Fractionated Radiotherapy

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**Abstract/Overview**
The National Cancer Institute's Radiation Research Program in collaboration with the Radiosurgery Society hosted a workshop on Understanding High-Dose, Ultra-High Dose rate and Spatially Fractionated Radiotherapy on August 20-21, 2018 to bring together experts in experimental and clinical experience in these and related fields. Critically, the overall aims were to understand the biological underpinning of these emerging techniques and the technical/physical parameters that must be further defined to drive clinical practice through innovative biologically-based clinical trials.

## Historical and current perspectives of Spatially Fractionated Radiation Therapy (SFRT)

Several SFRT or ultra-high dose rate experimental and clinical regimens are being studied pre-clinically with some early clinical experience available: GRID, FLASH, LATTICE Radiotherapy (LRT), and Microbeam Radiation Therapy (MRT). The common aims of SFRT or FLASH methods are to achieve better tumor control and/or reduced damage to normal tissue. Mechanisms might be shared, but currently not well understood. Bystander, abscopal effects, vascular damage, angiogenic and immuno-responses have all been proposed to be involved (**Fig.1**).

## Biology of SFRT

Research and clinical results from traditional GRID-type SFRT studies were the initial focus of the meeting. Patients with very large bulky tumors (≥ 6 cm) present a real therapeutic challenge to the treating physicians. These large tumors may be found at initial presentation or are also commonly present as recurrences in patients who have already received maximum surgery, chemotherapy or radiotherapy. Novel therapeutic approaches are needed in order to offer additional treatment options to these patients. The concept of GRID has been around for many years, developed as a means of reducing skin injury with the low energy X-rays available then. The idea was to place an absorbing grid directly on the skin of the patient, in order to reduce the dose to the basal cells of the skin directly under the grid. This grid would be out of focus a few millimeters further into the tissue and would therefore not affect the dose distribution in a deeper lying tumor (1). Yet it has not been studied in great detail as now used with high energy, deeply penetrating beams. Therefore, the possible biological mechanisms and extent to which GRID contributes to overall tumor control in the context of modern-day chemoradiation regimens are not clearly understood. While a number of provocative case studies were presented, there were limited or no comparative data presented between radiation therapy with and without SFRT, an aspect felt to be an important step in determining appropriate clinical application.

The opening session of the meeting was on biology, which aimed to demonstrate the biological underpinnings of various approaches utilizing spatial fractionation from laboratory experiments. Technology driven evolutions in radiation oncology were highlighted, which have enabled advanced conformal delivery techniques with dose delivery characteristics that share common features with SFRT on the scale of micron and centimeter wide beam diameters. *In vitro* and *in vivo* evidence supporting radiation induced signaling including bystander and abscopal effects, as potential mediators of response to SFRT were presented. The role of bystander effects was discussed as a potential explanation for SFRT responses followed by theoretical simulations of radiation-induced signaling for laboratory-based GRID beam geometries. Further evidence was presented demonstrating the biological effects of spatial fractionation on normal tissue vasculature and immune response along with clinical examples of radiation-induced signaling that included abscopal effects. In summary, current evidence using SFRT parameters scaled to fit experiments in the laboratory and not the exact patterns used in clinical practice geometry implicates multiple radiobiological

response mechanisms that need to be further studied, quantified and optimized to evaluate the efficacy and, as possible, develop the full potential of spatial fractionation for improving therapeutic index.

A number of data sets were also presented to describe spatial fractionation and the biological response of solid tumors to this type of radiotherapy. Preclinical studies employing a small animal conformal irradiation system have suggested that some of the biological mechanisms of action are related to bystander killing as well as vascular damage and re-modeling leading to improved tumor oxygenation (2-4). These aspects of biological response by the tumor have been studied in both aerobic and hypoxic conditions, and it was found that hypoxia modifies the bystander killing in some cases. In addition, it was observed that an inhibitor of angiogenesis could increase the effect of GRID and that the addition of thermal ablation to single dose GRID greatly improves tumor control in an orthotopic rat breast tumor model when added to a multi-modality therapy regimen (5) . Critical to these studies is the physical definition of the treatment technique, including the size of the higher and lower dose volumes with the SFRT field.

A special emphasis was given to Microbeam Radiation Therapy (MRT) as an experimental regimen as well. This approach of SFRT, which demonstrated better tumor control and normal tissue tolerance in animal models at very high doses, especially for brain tumors, had demonstrated that (bystander) induced differentiation played a role and could be used for benign conditions. In general, the orthovoltage mini-beam may have value due to the very high peak to valley dose ratios that can be obtained in comparison to high energy/high dose SFRT type of therapy.

In many cases advanced tumors are clinically radio- and chemo-resistant due to poor blood flow, hypoxia and low pH in the tumor. These factors may also influence the degree of bystander effects or immune surveillance generated by GRID therapy of the tumor. Clearly, large tumor volume has been found to correlate with decreased local control and survival in patients receiving conventionally fractionated chemoradiation for cancers such as head and neck, lung and breast. Thus, treatment modalities such as GRID radiation that can increase the tumor total dose and possibly activate beneficial bystander and abscopal immune effects while minimizing effects to normal tissues offer promising treatment options. Other data has suggested that there are abscopal/systemically expressed effects when SFRT is focally applied to a locally advanced tumor, and that they may be equal to or greater than those observed in standard whole tumor radiotherapy at high dose/fraction (6). Other groups have reported similar and fascinating trends in tumor immunity and control with partial radiation exposures (7,8). Older reports have demonstrated that GRID radiotherapy may induce TNF-$\alpha$, which might be an underlying mechanism for purported immunological effects (9). These studies need to be greatly expanded into multiple models and confirmed in clinical trials. The use of SFRT at present needs to be tested in patients receiving immunotherapy to evaluate the benefit of non-uniform fields on current cancer management practices. Taken together, related work from a number of different centers in the last 10+ years suggest a potential of safely and effectively including GRID or a related approach termed Lattice radiotherapy (described below)

against a variety of tumor types in combination with standard of care regimens to maximize tumor control (10-14).

**Technical and clinical implementation of SFRT**

SFRT by definition, divides treatment volume into fractional sub-volumes with high and low doses alternating between the sub-volumes. Accordingly, GRID (the first type of SFRT introduced in early 1950s, (15), LATTICE and MRT are all considered as SFRT. While MRT is still at the stage of preclinical study, GRID and LATTICE have been implemented clinically. This section will focus on the technical and clinical aspects of GRID and LATTICE therapies.

The early inception of GRID therapy was based on the objective of sparing normal tissue especially skin, while delivering an effective palliative dose to bulky tumors in the early days of orthovoltage therapy. GRID therapy being applied clinically with megavoltage beams uses a grid-pattern radiation fluence, which is created by the use of either a grid physical block (commercially available block) (16) software generated virtual block, or a multi-leaf collimator (MLC) modulation (17). Following the convention of early GRID orthovoltage practice, the size of grid openings (peaks) and the separation (valleys) are typically near 1 cm. Proton beams have also been used to deliver GRID therapy, with similar spatial arrangements (18). Traditionally, GRID therapy refers to peak-valley distributions arranged in 2D. LATTICE is a 3D extension of traditional GRID treatment, in which, a number of high dose regions (sphere-like vertices that are planned to receive in the range of 15-20 Gy) are separated by low dose regions (19). The size of high dose vertices and distance between them closely resemble that of GRID, although not rigorously adhered to. No normal tissue outside of tumor volume will receive high dose as part of the treatment planning goals for LATTICE. LATTICE radiation therapy (LRT) can be delivered by photon-based IMRT, VMAT, robot-driven converging beams, or by charged particle beams.

For the past decade, GRID therapy, although not a mainstream treatment modality, has been used by a number of clinicians to treat many patients who they consider would have otherwise been denied other treatment options (20) and they are encouraged by their observations. Mohiuddin et al. (21) reported that among 71 patients and 87 sites treated, presenting with a variety of cancer types treated with GRID therapy with 15 Gy peak dose as a goal there was a 78% response rate for pain and no grade 3 toxicities were observed in any patient. When GRID therapy was followed by conventionally fractionated external-beam therapy (open-field), a clinical complete response was seen in 5/8 patients and pCR was found in 50% of resected specimens. The complete response in patients treated with an open-field external-beam radiation doses of 40 Gy or greater was higher than that seen in patients treated with lower doses. Mohiuddin's experience indicated that GRID therapy applied before open-field treatments appears to significantly and safely improve the response of tumors in a dose dependent manner, but such observations require carefully controlled trials for wider validation of the approach.

LRT, although relatively new, has already been applied to over a hundred patients in a number of clinical indications, including cervical squamous cell carcinoma, ovarian, and non-small cell lung cancer with excellent local control and minimal toxicity when delivering a single LRT up to 18 Gy to the vertices and around 3 Gy to the surrounding volume between each vertice (12,13). Currently, the dosimetric consideration of photon-based LRT falls generally in the category of IMRT, VMAT, and stereotactic body radiotherapy (SBRT). In LRT, the dose is prescribed to the maximum dose of vertices. The cumulative empirical clinical experience presented at the workshop was a start to developing useful guidelines to begin a new LRT program which requires standardized definition and terminology to allow for multi-center trials with well-defined prescriptions, a goal of this workshop.

The dosimetry of clinical GRID treatment is different from conventional RT and has been carefully studied and published (16,22). With the assistance of experienced physicists from a number of clinical centers having active GRID programs, implementing GRID therapy is readily feasible. The dose of GRID therapy has been traditionally prescribed to the depth of maximum dose along the beam path. Furthermore, with advancements in the physical parameters of GRID and LATTICE (size of grid, peak-valley dose ratio, etc.) additional studies are needed to validate and optimize these parameters at the biological level. Accordingly, technical improvements of GRID/LATTICE will rely heavily on progress in radiobiology and systematic clinical studies. Today, there are few clinical trials of GRID therapy (23) and given the empirical and encouraging clinical data, there is an urgent need for more systematic clinical studies. In recognition of the lack of uniformity in historical clinical practice of GRID/LATTICE, a major emphasis has been placed on standardizing the physics and dosimetric aspects of the techniques, as well as in defining clinical endpoints. But before we can proceed to clinical trials, there needs to be consensus on the dose, fractionation and physic quality assurance process for GRID/Lattice therapy based on current knowledge of this approach across the different institutions. Moreover, agreements on the clinical indications that would likely best benefit from this SFRT will help guide the design and focus of clinical trials. An active working group has been established to address these points.

**Clinical trials for ultra-high-dose rate or SFRT**

The clinical progress made in GRID, LATTICE and synchrotron-generated microbeams was reviewed, with an aim of discussing potential multi-center clinical trials that can and should be pursued in an attempt to reduce normal tissue toxicities and/or even improve tumor control.

SFRT is an attractive treatment approach that enhances normal tissue-sparing and has encouraging observations in otherwise challenging palliative care situations. Future clinical trials of SFRT should investigate its differential effect on the immune system. Specifically, single fraction delivery of high dose irradiation, as is performed for SFRT or other more mainstream approaches like SABR, may have immunological effects that differ substantially from conventional fractionation (24). Alternatively, larger doses per fraction may impair immune response (25). Furthermore, the peak-valley differential

dose effect could potentially optimize immunological processes that can result in abscopal effects (26). Proton-based SFRT may have its own immune benefits while also allowing for absolute fall off of irradiation dose distal to the target volume. In that induction of immune response can vary for the different target tissues (tumor, vasculature, immune infiltrate, stroma, etc). the timing of SFRT, use of one of multiple fractions, timing between SFRT and open field, etc, may have a critical impact on optimizing efficacy and of using this with immuno-modulators.

Synchrotron-generated microplanar beams (microbeams) are another attractive modality to test, due to its minimal beam divergence allowing for extremely sharp dose deposition along the beam path, which can facilitate hundreds to thousands of Gy to be delivered to microscopic tissue slices while dramatically reducing the risk of radionecrosis (27). This limited toxicity detected is attributed to the relative radioresistance of normal large vessels to high doses delivered in microbeams and the rapid regeneration of normal microvessels. Moreover, spatial fractionation delivered with this modality allows for valleys of very low dose regions (28). Microbeam treatment of solid tumors allows the delivery of doses up to 1000 Gy with selective damage to the tumor and general preservation of healthy tissues (29). Microbeam radiosurgery has been investigated in vivo to ablate or transect selection central nervous system structures thought to contribute to such disorders like Parkinson's Disease, Huntington's Disease, and seizure disorders (30). Aside from the tight dosimetry, the low energy of monochromatic beams makes them well-suited to treat superficial targets within the brain. MRT is currently being pursued in the preclinical settings, where it is highly anticipated that clinical indications ranging from cancers to functional brain disorders will be evaluated for upcoming clinical trials.

**Brachytherapy and High Dose Rate versus GRID/Lattice/Flash: How can we exploit the understanding and impact of GRID/Lattice based on the success of brachytherapy in the clinic?**

Potential parallels and synergies between GRID/Lattice therapy and brachytherapy (BT) were explored. The most obvious similarity between these two treatment approaches is the inherent heterogeneity in dose distribution, particularly with interstitial BT, where "peak" and "valley" doses commonly show a 4- to 6-fold difference.

Clinical evidence supporting the use of monotherapy or combinatorial BT has shown high rates of tumor response and disease control, and this appears mirrored by the preliminary evidence supporting the use of GRID/Lattice therapy. Although it can be argued that these improvements are entirely a consequence of the dose-escalation achieved with BT, the panel felt that the dose inhomogeneity of BT may play a role. For example, evidence in prostate cancer has suggested that conventional dosimetric parameters of overall BT dose are insufficient to explain the variability in outcomes (31). The study of this heterogeneity could uncover relevant biological mechanisms. Similarly, in cervical cancer the recent use of image-guided BT has been associated with higher local control rates and potentially improvements in survival compared to historical outcomes using intracavitary-only approaches (32,33). These advancements in outcomes could be in part explained by a better definition of the target volume and

enhanced dose coverage. However, considering the increased use of interstitial BT component in over 40% of image-guided BT treatments (32), the higher dose-heterogeneity from the increasing interstitial component may also contribute to the observed improvements in outcomes.

While many parallels exist between interstitial BT and GRID/Lattice therapy, the "peak-to-valley" dose differential is generally greater and steeper in BT compared to GRID/Lattice dose distributions although further determination of the dose delivered versus dose-planned for GRID/Lattice will be informative. Moreover, the impact of motion on GRID/Lattice may smooth out dose heterogeneity while BT should hold their different peaks and valley dose differentials. Similarly, the optimal valley dose (across the entirety of the tumor), the extent and spatial distribution of dose inhomogeneity, and additionally the temporal distribution of dose (time interval between treatments) are yet to be determined.

High-precision anatomical and functional/molecular imaging is an important component of tumor response assessment and integral to mechanistic studies of understanding and optimizing SFRT. Anatomical imaging precisely quantifies volumetric tumor response. The volumetric parameters can be augmented by imaging assessment of functional/biologic tumor characteristics, such as tumor microcirculation, hypoxia, cellularity and metabolic activity based on various molecular imaging tracers, and can correlate spatial dose distributions with the spatial metrics of functional tumor response (34). These functional/molecular tissue properties are distributed heterogeneously throughout the tumor volume. Therefore, in the context of heterogeneously delivered radiation doses, incorporation of functional/biologic and anatomic imaging provides a unique opportunity to longitudinally assess changes non-invasively, providing insight into tumor properties on a sequential whole-tumor scale.

Tissue correlation of tumor biopsies at BT and dose correlation may allow BT to serve as a good test bed for molecular correlates in Lattice/GRID. While tumor tissue collection for translational research has been challenging in solid tumors, BT uniquely enables correlative translational studies to interrogate direct and indirect effects of radiation (e.g. molecular mechanisms of abscopal, bystander, vascular, and immune responses). These correlates may help elucidate, in the context of clinical trials, an improved understanding of the role of immunomodulation and priming of immunotherapy (6). Tumor biopsies can be seamlessly integrated into a BT procedure, and tissue samples correlated with high resolution anatomic and functional imaging (35), including spatial variations in dose. Moreover, tissue collection can be obtained repeatedly in fractionated high dose rate procedures for longitudinal assessment of molecular tissue correlative markers (36). Co-location of these tissues correlates with the functional/molecular imaging parameter maps of cellularity (DWI MRI), vascularity (DCE MRI, DCE CT) and molecular tumor biomarker properties (PET tracers of glycolysis, proliferation and others) (34), which are now entering the BT paradigm (37) will be crucial. In turn, robust and validated non-invasive imaging biomarkers could be translated into clinical studies to refine treatment planning and response assessment.

Clinical trials leveraging BT for the exploration of GRID/Lattice therapy hypotheses should rely heavily on clinical outcome endpoints (or validated surrogates), such as quantitative tumor response, metastasis-free and overall survival rates, and strong translational components through tissue correlation pre-, post-, and potentially during therapy for longitudinal assessment. Incorporation of functional/molecular imaging-based response assessment of vascular and metabolic effects can be considered to advance our understanding of molecular mechanisms in spatial/temporal heterogeneous dose delivery in patients. The potential for assessing tumor and immune response through circulating biomarkers will be critical for SFRT and for integration of radiation oncology in immuno-oncology progress.

**Biological effects of FLASH-RT and MRT**

Ionizing radiation provides an effective means for killing mammalian cells, and the field of radiation oncology specializes in using this physical agent to preferentially kill cancer cells while minimizing adverse outcomes to the surrounding normal tissue. This session was devoted to rationalizing the potential benefits of two non-traditional irradiation modalities, namely MRT and FLASH-RT. The use of MRT has received attention over the years due to the ability to modulate the spatial distribution of dose, based on the dramatic “peak and valley” dose differentials that can be obtained with GRID (29,38). The emergence of ultra-high dose rate irradiation known as FLASH-RT implements mean dose rates in excess >40 Gy/s, delivered as a single or a few pulses over intervals of milliseconds (39) . While past reports have referred to mean dose rates, this in fact may be inadequate to properly describe the necessary FLASH parameters, as discussed further below. In any case, these variations in beam delivery are in marked contrast to more traditional clinical irradiation modalities that implement more homogeneous irradiation fields typically delivered at much lower dose rates (~0.03 Gy/s) and over the timeframe of minutes. Session participants focused on the differential impact of MRT on the vasculature, and the preferential sparing of normal tissue complications without compromising tumor control by FLASH, features that are discussed further below.

**MRT**

The main limiting factor to further increase therapeutic radiation doses and the related tumor control for patients with cancers of unmet clinical need is the severe toxicity induced in vital healthy tissues by the treatment itself (40). Synchrotron X ray microbeams have been recognized since the 1980s as a unique therapeutic opportunity to overcome this dose limitation (41). This enticing possibility has elicited scientific interest across the world in radiotherapeutic applications of microbeams, referred to as MRT. MRT consists of a spatially modulated microscopic dose array of low energy X-rays of 50 to 300 keV delivered at exceptionally high dose rates (up to 16 kGy/s). These spatially fractionated microscopic beams exploit the dose-volume effect leading to extraordinary normal tissue tolerance that triggers an entire cascade of differential biological effects able to improve tumor control.

Decades of careful pre-clinical studies have consistently demonstrated the therapeutic efficacy of MRT in different tumor models such as gliosarcomas, squamous cell

carcinoma and breast carcinoma (29,42-44). Normal tissues, which included the brains of adult rats, the caudal fin of zebrafish, immature tissues in suckling rats, duck embryos, the chick chorioallantoic membrane (CAM) and piglets have all showed a resistance of normal tissues to micro-beam irradiation applied in the MRT mode when compared with conventional uniform field irradiation (38,45-49). Importantly, a long term study using the normal mouse ear pine (containing many types of tissues) demonstrated clearly that normal tissues such as skin, glands, cartilage, blood and lymphatic vessels are highly tolerant to MRT after entrance doses up to 800 Gy (50).

Unpublished data in a study initiated by Varian Medical Systems (Palo Alto, CA, USA) indicated that MRT induces only minor patchy pulmonary fibrosis one-year post irradiation, following peak entrance doses of a few hundred Gy. MRT has the potential to control lung tumors with minor side effects, currently one of the major medical challenges in radiation oncology. While the pre-clinical evidence obtained in animal models strongly supports the potential clinical benefits of MRT questions do remain. The ultimate implementation of MRT as a new paradigm for cancer care will be facilitated by a deeper understanding of the underlying biological mechanisms responsible for the differential effects of MRT between tumors and normal tissue. Elucidating the relevant molecular pathways will help identify the most effective treatment protocols for future clinical trials.

Recently performed studies using murine tumor models and *in vivo* CAM and zebra fish fin assays demonstrated that MRT is able to disrupt the immature (tumor) vessels by preserving the normal vasculature of the surrounding tissue (47). In addition, MRT triggered an acute inflammatory response (eventually also a late immune response) restricted to the immature tumor vessels or regenerating tissue (49). Those data indicated that MRT can be used as a novel angiodisruptive cancer treatment strategy. MRT represents a simple and unique method that can destroy immature tumor-vessels while sparing mature normal-tissue vessels from radiation damage. When the dose delivered is in the range of 100-300 Gy, MRT causes a partial disintegration of the endothelium leading to a temporal but significant increase in tumor blood vessel permeability. We termed this phenomenon “MRT-induced vascular permeability window,” and it has been identified as potentially important for drug delivery. Finally, the MRT-irradiated blood vessels could serve as a homing beacon for circulating immune cells, thereby possessing the potential to modulate the anti-tumor immune response.

**FLASH-RT**

Ultra-high dose rate irradiation (in sub-second time scales), coined “FLASH-radiotherapy” (FLASH-RT) by Favaudon *et al*. in 2014 (39), has gained attention because of the surprising observation in preclinical studies of model *in vivo* systems finding markedly increased therapeutic index compared to conventional dose rate irradiation that is delivered over minutes. Importantly, the ultra-short time of radiation delivery distinguishes the FLASH-RT modality from current radiotherapy. It saturates the irradiated tissue bed within microseconds producing an instantaneous burst of free radicals whereas irradiation at conventional dose rates produce free radicals in a more chronic manner transpiring over minutes (51,52). This initiates a series of reactions that

are qualitatively similar but quantitatively distinct so as to elicit markedly different biological responses to the initial deposition of damage that remains linear with the absorbed dose (53). In fact, normal tissue sparing by FLASH has been described at least as far back as 1966 (54), attributed to the depletion of oxygen in tissues at very high dose rates (55), but in the last few decades this line of research has been relatively dormant until this recent renaissance of interest. While the precise mechanisms underlying the differential response of tissues to FLASH-RT remain to be elucidated, careful dosimetry using 4 distinct calibrated systems have indicated clearly, that the measured differences in biological response observed between FLASH and lower dose rate delivery systems is simply not the consequence of different absorbed doses (56-59). Importantly, if not remarkably, the beneficial effects of FLASH-RT were first elucidated in vivo using preclinical mouse models (39,58), rather than using in vitro cell systems or more simplistic model organisms such as yeast, worms and flies.

Normal tissue toxicities generally limit the maximum dose that can be delivered to the tumor bed without compromising patient safety (40,60,61). These empirically derived relationships have evolved over years of clinical experience and define the dose limits that can be and have been modulated through changes in fractionation schedules and access to IGRT. Clearly, any strategy capable of minimizing normal tissue toxicity has the potential for promoting dose escalation to the tumor bed, increasing the chances for local regional control and progression free survival. Since the seminal paper by Vozenin and colleagues (39), FLASH-RT has been shown to dramatically spare normal tissue toxicities in multiple organ sites including, the lung, gut, skin and brain (58,62-64). Importantly, in late responding tissues, adverse functional outcomes such as lung fibrosis and neurocognitive decline typically found after conventional radiotherapeutic regimes can be minimized or even avoided when radiation doses are delivered at ultra-high dose rates.

The phenomenon of the increased therapeutic index of FLASH compared to conventional dose rate irradiation, or the “FLASH effect,” has now been reported in multiple preclinical models. Normal tissue sparing by FLASH of multiple organ systems including lung, brain, intestinal tract, and skin has been demonstrated in multiple mouse strains and even additional species (cat and mini-pig), while equal (and in some cases superior) tumor killing between FLASH and conventional dose rate has been observed across multiple *in vivo* tumor models (58,62,63). The burgeoning field of FLASH-RT is now populated with more recent reviews than solid data driven manuscripts, and some confusion has emerged concerning the optimal beam parameters required to elicit the “FLASH effect”. To remedy further and unnecessary uncertainty, it seems logical that authors claiming to evaluate FLASH-RT, should be required to provide the critical beam parameters used in a given study. Without this information, as was the case in a recent report claiming a “negative” FLASH effect (65), it is virtually impossible to properly evaluate any conclusions drawn and place them in proper context within this expanding and evolving discipline. Indeed, the group in Lausanne Switzerland led by Dr. Vozenin has suggested just such course of action, and has stressed that quoting mean dose rates may be an oversimplification of the important time signatures of a pulsed FLASH

beam. In this light, a more accurate definition of the ultra-high dose rate parameters needed to trigger the FLASH effect is presently defined in terms of instantaneous intra-pulse dose rates (≥ $10^4$ Gy/sec) and overall time of exposure less than 100 milliseconds (*i.e.* ≤ 0.1 sec) (66).

Given the nascent state of the field, a large portion of experimental observations to date remain preliminary and unpublished, and many questions remain unanswered particularly with respect to mechanism. While there are data suggesting a fundamental physical-chemical effect (*i.e.*, radiochemical depletion of oxygen at FLASH dose rates), modulation of inflammatory cytokines (*e.g.*, TGF-β) and differential immunologic responses between tumor and normal tissues have also been observed, which might be either downstream effects or independent mechanisms. Provocative data presented at the workshop showed that FLASH irradiated mice bearing orthotopic brain tumors exhibited improved performance on a learning and memory task compared to mice subjected to conventional dose rate irradiation. Importantly, the neurocognitive benefits afforded by FLASH-RT relative to standard dose-rate RT were substantiated further using a variety of learning and memory tasks and extended to reductions in anxiety, depression and improved fear extinction. These findings demonstrated long-term and persistent (6 month) benefits that could be achieved with FLASH-RT, thereby avoiding the debilitating neurocognitive complications normally associated with conventional dose rate irradiation. Additional data sets pointed to several potential underlying mechanisms able to explain the FLASH effect, and included reduced neuroinflammation and a preservation of host neuronal morphology. Certainly, we have barely scratched the surface of potential mechanistic pathways to be investigated. These normal tissue benefits portend a change in the field of radiation oncology, which necessitates a consideration of the effects of FLASH-RT on tumor control among other topics as discussed further below.

**FLASH – challenges, issues, strategies and hurdles for clinical trials**

The clinical relevance of FLASH has been limited by the lack of practical technologies to deliver such rapid treatments to human patients with typically deep-seated macroscopic tumors. However, this is poised to change with recent advances in linear accelerator technologies. Thus, the question of how to implement clinical trials for translation of FLASH is ripe for discussion. In this light, an improved understanding of mechanisms from preclinical studies would inform more optimal designs of clinical trials. While this is being studied using in vitro and in vivo models, clinical trials can now be considered. With electron-based delivery, the first clinical trials may be most ideal if performed in small to moderately size tumors in locations amenable to surgical salvage should incomplete tumor control be achieved with FLASH or there is a need for procedural intervention of a radiation-induced toxicity. Superficial targets may also have additional translational endpoint benefits, including allowing for serial biopsies for histologic, genetic, and immunologic analyses, as would targets being treated preoperatively. Proton-based delivery may allow for additional sites to be considered for clinical trials. Very recently, the group at the University of Pennsylvania has described the development of a proton-based system for delivering FLASH-RT, which will prove useful

in defining further the limits and capabilities of this innovative radiation modality (67). To date, very little data on FLASH exist outside of single large fractions, and the utility of fractionated FLASH therapy or the combination of FLASH with chemotherapy or immunotherapy is not well characterized, and additional data with these approaches are needed before clinical trials. Thereafter, if determined safe and feasible in humans, future randomized phase 2 trials could be considered comparing FLASH to conventional dose rate SABR and/or conventionally fractionated radiotherapy for selected indications, particularly for tumors where there is currently a high incidence of measurable toxicity with the best standard of care.

It should be emphasized that the capability of FLASH-RT to spare normal tissue complications must be tempered under the possibility that such protective effects might also extend to the tumor, a clearly undesirable outcome. This topic was addressed directly during the workshop where data derived from three independent tumor models showed that single fraction doses of either FLASH or conventional dose rate irradiation were equally efficient at delaying tumor growth (Montay-Gruel, unpublished data). Flank tumors, spontaneous tumors and orthotopic brain tumors of relevance to glioblastoma multiforme (GBM) were all equally responsive to either irradiation modality (Montay-Gruel, unpublished data). Data shown demonstrated convincingly that FLASH-RT did not spare the tumor, but rather, was isoefficient at tumor control when compared side-by-side with conventional dose rate irradiation. While additional investigations using higher tumor control doses delivered as either single or multifraction regimes using FLASH-RT are clearly warranted, data to date does NOT indicate that tumor control is compromised by FLASH-RT.

Moreover, the FLASH effect is incompletely characterized even from a phenomenological standpoint. Evidence to date suggests that a dose rate threshold of approximately 40 Gray/second or higher is needed to produce the FLASH effect, with potential improved normal tissue sparing at even higher dose rates (39,58). However, the accelerator-based radiation delivery systems used for FLASH experiments to date produce pulsed radiation, and there are many aspects to delivery speed – total dose and delivery time, dose per pulse, pulse timing structure, etc. – that may be critical to the effect and have not been comprehensively evaluated, since an exhaustive study is an experimentally daunting combinatorial problem. Clinical radiation therapy is predominantly fractionated, but nearly all research on FLASH to date has been with relatively large single fractions. As such, demonstrating the FLASH effect in fractionated schedules has important clinical relevance. Similarly, as much of clinical radiation therapy across disease sites is given as part of combined modality cancer treatment, an important question is whether the FLASH effect holds up in the context of systemic therapies. Such experiments are only starting to be done.

A major practical limitation to clinical translation of FLASH is technological. To date, preclinical FLASH irradiation of small animals and small, superficial targets in larger animals has been possible using modifications of existing irradiation systems that are capable of producing FLASH dose rates when limited to small volumes (of up to a few cubic centimeters), including electron linear accelerators (56,68), a synchrotron light

source producing kilovoltage energy x-rays (69), and certain proton accelerators (67,70). However, recent advances in linear accelerator science and radiofrequency power generation and distribution technologies have led to prototypes of compact, high-efficiency linear accelerators suitable for producing high-energy x-rays (in the megavoltage energy range) or very high-energy electrons (exceeding 100 MeV energy) capable of treating large-volume, deep-seated targets at FLASH dose rates. In particular, Stanford University and SLAC National Accelerator Laboratory are engaged in a program to develop a medical linear accelerator system based on these technologies, called pluridirectional high-energy agile scanning electronic radiotherapy (PHASER), designed to deliver highly conformal FLASH intensity-modulated radiation therapy to general radiation therapy targets (71). The technical motivation for developing this technology was as a fundamental solution to the problem of uncertainty owing to organ motion, by combining rapid volumetric image-guidance and treatment delivery that could be completed faster than the time scale of physiologic motion. The same delivery speed would enable clinical evaluation of FLASH biological effects.

Can clinical testing of FLASH begin without a complete understanding of its biological mechanisms of action? A better understanding of mechanisms from preclinical studies would likely inform more optimal designs of clinical trials. Arguably however, it is possible to design some rational clinical trials without extensive understanding of mechanisms – indeed, our mechanistic understanding of even our current standard of care treatments is far from complete. Moreover, with thoughtful design of translational endpoints, clinical trials will facilitate our mechanistic understanding of new treatment modalities such as FLASH and ultimately may be essential for it.

Considering trials that could be done with slight modifications of existing technology, one example strategy would be to start with phase 1 trials of FLASH electron beam therapy for superficial tumors such as skin cancers, including selected squamous cell, basal cell, or melanoma cancers. Ideal tumors to evaluate would be those of small to moderate size and location amenable to surgical salvage (in the case of incomplete tumor control) and/or reconstructive surgery or skin grafting (in case of late skin injury or radionecrosis). In this setting a reasonable strategy would be to test single fraction or hypofractionated FLASH regimens starting at doses high enough to produce relatively high tumor control probability and moderate risk of skin injury based on conventional dose rate data, such as 20-25 Gy single fraction. This clinical scenario lends itself readily to collection of translational endpoints such as serial biopsies for histologic, genetic, and immunologic analyses. Alternatively, FLASH radiotherapy could be administered preoperatively to skin tumors planned to undergo definitive surgical resection to assess biomarkers of tumor response, normal tissue injury, and inflammatory/immunologic responses. In fact, a phase 1 trial of electron beam FLASH for skin lesions has already been initiated at Lausanne University, and results of their first patient treated for T-cell cutaneous lymphoma have recently been published (72).

The near future implementation of technology such as PHASER or proton therapy cyclotron-based FLASH that would allow delivery of radiation to a full range of target volumes with current state-of-the-art conformity but at FLASH dose rates would afford

unique opportunities for randomized clinical trials. Historically, trials comparing different radiation delivery modalities, for example IMRT *vs*. 3-D conformal RT or proton *vs*. photon, have been hampered by confounding variables because the fundamentally different dose distributions being compared could produce outcomes that were unanticipated prior to insights gained from more mature clinical experience. For example, in the early days of IMRT for head and neck cancers, an exclusive emphasis on parotid gland sparing led to plans with increased oral cavity dose and concomitant toxicity. In this way, engaging too early in randomized clinical trials of a new modality before understanding its optimal use risks prematurely concluding a lack of benefit or even seemingly proving a detrimental effect. However, in the case of FLASH, given a delivery system that can produce high quality conformal treatments, it would be possible to compare FLASH *vs*. conventional dose rate plans that are identical in every way – dose and fractionation, target delineation and margins, dose distribution (identical dose-volume histograms for every organ), and the skill and effort of the planner – *except* for speed of delivery, isolating that as the only variable. After creating one optimized plan, randomization could be simply on whether FLASH is "switched on."

With such a capability, it would be possible empirically to test whether FLASH provides an advantage in different clinical scenarios. A reasonable approach would be to conduct randomized phase 2 studies using standard of care regimens, including stereotactic ablative (SABR) and conventionally fractionated radiotherapy (CFRT), for selected indications. Particularly attractive settings would be those in which: (1) there is currently a high incidence of measurable toxicity with the best standard of care, *e.g.*, concurrent chemoradiotherapy for head and neck cancers or pediatric brain tumors in which whole cranial irradiation is used; (2) there is a high incidence of otherwise readily measurable early or delayed tissue damage, *e.g.*, lung fibrosis on CT imaging after SABR; (3) accessible anatomic sites (by endoscopy or direct visualization, *e.g.*, bulky cervix cancer) amenable to serial biopsies for mechanistic studies. The initial focus should be on scenarios for which there is the potential to observe a large effect size, especially on normal tissue sparing, should one exist. Equipoise is facilitated in that the control arm would be the best current standard of care, there is no suggestion from preclinical or even historical clinical data of an adverse effect of higher dose rates, and the technical objective of improved motion management through rapid treatment is valid independent of potential biological benefits.

In conclusion, FLASH has the potential to be a paradigm shift in curative radiation therapy with preclinical evidence of fundamentally improved therapeutic index. While much remains to be understood about the mechanisms underlying the phenomenon, a rational approach to initial clinical testing is possible, and will contribute to a mechanistic understanding. Elucidation of the mechanisms through preclinical and clinical translational research will inform more specific and innovative clinical trial designs in the future, such as optimal combinations with oxygen modulation, immunotherapy, or other potentially biologically synergistic approaches.

**Clinical trial design and comparative effectiveness.**

Technological advances in radiation oncology treatment planning and delivery have generally not been tested in comparative effectiveness trials but have been marketed based on a so-called 510(k) pre-market clearance from the U.S. Food and Drugs Administration (FDA). However, the novel radiation delivery methods discussed here are different: SFRT and FLASH-RT involve novel biological mechanisms that will need to be tested in well-designed controlled clinical trials before being widely adopted in routine clinical care. These innovative radiation therapy methods are no different from the biological hypotheses tested in the large time-dose-fractionation trials successfully conducted over the last 25 years in many tumor sites (73), or the combined modality trials of chemo-radiation therapy or radiation-immune-oncology combinations versus radiation therapy alone.

Once the clinical target volume is defined, a traditional course of radiation therapy can be described using only a handful parameters such as radiation modality, total dose, dose per fraction, overall treatment time, and some indication of dose uniformity in the target volume. In contrast, SFRT involves many more degrees of freedom for example the peak-to-valley dose contrast, the spatial frequency of peaks and valleys, the three dimensional 'lattice' of dose peaks delivered. The relative biological importance of these metrics and how these will interact with dose-fractionation in the temporal domain as well as with other anti-cancer modalities remain to be defined. One obvious issue that needs clarification is whether a spatial shift of the dose-lattice from one fraction to the next would essentially negate the effect. Similarly, FLASH-RT has typically been delivered as a single fraction in the pre-clinical setting. This would essentially make the 4R's of radiotherapy (repair, reoxygenation, regeneration, and redistribution) irrelevant. However, from a clinical perspective, the relevant comparator would arguably be a fractionated, possibly hypofractionated, schedule delivered at standard acute dose-rate. Whether the "FLASH" effect gradually disappears with decreasing ultra-high dose rate is not clear. With all these delivery methods, carefully planned and conducted preclinical in vivo experiments will undoubtedly be required to develop a rational roadmap for preclinical to clinical translation.

Several of the challenges in dosimetry, quality assurance, radiobiology, and clinical case selection have been discussed above. From a statistical design perspective, the most obvious consideration is that the relevant patient-level benefit must be quantified in terms of a therapeutic ratio, i.e. a risk-benefit estimation. While some of these methods have been introduced in a compassionate way in the treatment of single cases, the systematic first-in-man trials should be designed as phase I/II dose escalation studies. The aim of these studies would be to establish a recommended schedule for a subsequent comparative-effectiveness trial with standard radiation therapy as the comparator. Because of the large patient-to-patient variability in outcomes, the early phase trials should preferably be randomized. Adaptive trial designs with relatively rapid dose titration may be attractive. While the aim of the early trials would be to establish a radiation dose that achieves isoeffectiveness with respect to adverse events compared with standard radiation therapy, a – possibly relaxed – stopping rule related to efficacy should be enforced for patient safety. This trial will need to have a sufficient sample size to arrive at a reasonably accurate estimate of the

Recommended Phase 3 Dose (RP3D), c.f. the RP2D concept used in phase I drug trials.

Phase III trials with dual primary endpoints will be required to show a meaningful benefit in terms of therapeutic ratio: demonstrating superiority with respect to toxicity and at the same time non-inferiority within a given margin in terms of tumor control; or, alternatively, superiority with respect to tumor control and non-inferiority with respect to late adverse effects. A trial powered to show sparing of adverse effects is not going to be conclusive unless it also has the statistical power to show that this normal tissue sparing can be achieved without an unacceptable loss of efficacy.

Clinically relevant non-inferiority and superiority margins are required to make the trial outcome practice-changing. This will require large sample sizes that are only achievable in large co-operative group trials, possibly inter-group trials across many of the established clinical research networks.

**Overall summary and conclusions**

Progress in science depends on change and innovation, and the field of radiotherapy is no exception, as it has potential advancements in physics, chemistry and biology all aimed at improving the therapeutic index. This report has highlighted some alternative dose delivery modalities able to exploit certain differences between normal tissue and tumors that have resulted in some rather unexpected beneficial effects now substantiated in multiple labs (FLASH) and empiric observations for other SFRT techniques. While the detailed description of how volume sparing in the “valley” region of MRT promotes tumor kill and normal tissue recovery awaits further investigation, present results point to a fascinating paradigm with potential considerable clinical promise. Similarly, FLASH-RT explores a different range of dose rates compared with the ‘traditional’ dose-rate effect, where decreasing dose rate is associated with an improved therapeutic ratio. For this and other reasons, FLASH-RT has caught the field by surprise, where a relatively straightforward change in beam delivery has led to significant improvements in the therapeutic index, heretofore unrealized by other advancements in medical physics. One fascinating observation is that pencil beam proton GRID techniques might blend the advantages of both SFRT and FLASH (since the dose-rates at the distal edge of the beamlets might be in the FLASH dose-rate range) (74). Table 1 is a primer and quick reference/summary of this meeting report and the discussion to date by members of the program and assembled working groups regarding the state of research and translation potential for each sub-area of the broader movement to find innovative uses for ionizing radiation.

The capability of FLASH-RT to ameliorate normal tissue complications in the brain as well as several other organ sites while exhibiting isoefficient (or potentially even improved) tumor control has stimulated significant excitement in the field, and is poised to change the landscape of current practice in radiotherapy. While beam and pulse characteristics along with protocols for delivering single versus fractionated FLASH-RT remain to be fully optimized, these advancements have defined new avenues for the field and should be pursued with judicious and rigorous research (75). While the need for accurate dosimetry and precise dose delivery have always been at the forefront of

clinical safety and practice, other concerns revolving around these new modalities should not stifle their development. As a recurrent theme that remains relevant, change is continually needed for the optimal advancement of new technologies that aim at improving health care.

Presented at the workshop and published separately from this report (due to the need for information from NCI workshops to be available within a year) is an editorial regarding implementation of new technologies in routine clinical practice (68). As noted, this workshop has led to the establishment of physics, biology and clinical working groups that can take the enthusiasm, observations and data forward into the necessary studies for appropriate clinical implication for radiation oncology alone and in combination with drugs and immune-modulators.

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## Figure Legend

**Figure 1.** Proposed biological mechanisms of SFRT

Pros/Cons and current state of knowledge for spatial fractionation and ultra-high dose rates

| | GRID/Lattice | FLASH | Microbeam |
|---|---|---|---|
| ***Pros*** | | | |
| **Reduction in treatment #**<br>Evidence level/translational potential: | Yes/Medium | Yes/High | Yes/? |
| **Normal tissue sparing**<br>Evidence level/translational potential: | Yes<br>Multiple clinical studies | Yes<br>Several pre-clinical reports (multiple models). One clinical case report | Yes<br>Numerous pre-clinical reports, large animal studies |
| **Increased clinical response**<br>Evidence level/translational potential: | Yes<br>Multiple clinical reports | Medium<br>One case report | ?<br>Little to no clinical data |
| **Mechanisms of action understood**<br>Evidence level/translational potential: | Partial<br>Medium | Partial<br>High | Partial<br>Limited |
| **Pro immune function**<br>Evidence level/translational potential: | Suggested<br>pre-clinical | Medium<br>? | ?<br>pre-clinical |
| ***Cons*** | | | |
| **Limited use/applications**<br>Evidence level/translational potential: | TBD/<br>Dedicated clinical studies needed | TBD/<br>Data on larger treatment volumes needed | Likely/<br>Tougher to implement clinically,<br>Limited facilities |
| **Difficulty to obtain technology**<br>Evidence level/translational potential: | NO | Maybe/<br>Higher beam energies and field sizes needed | Yes/<br>Synchrotron based |
| **Cost**<br>Evidence level/translational potential: | Low | Med/high | High |
| **Site access (deep seated/superficial)**<br>Evidence level/translational potential: | Options growing | Not known/<br>superficial confirmed | Yes/<br>Numerous brain studies |

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| **Pro immune function**<br>Evidence level/translational potential: | Suggested<br>pre-clinical | Medium<br>? | ?<br>pre-clinical |
| ***Cons*** | | | |
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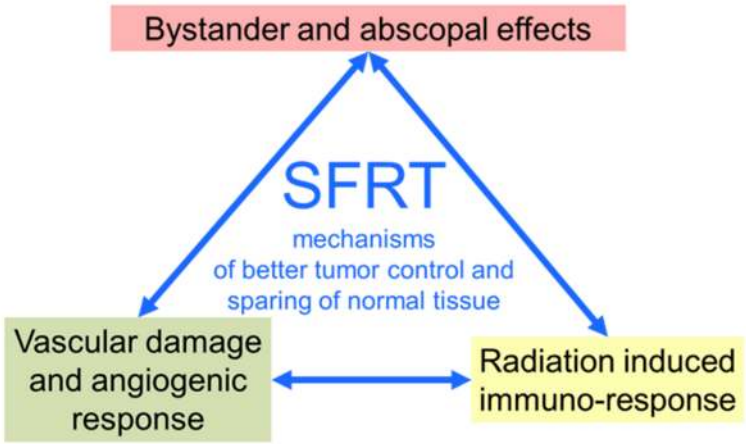
Bystander and abscopal effects
SFRT
mechanisms
of better tumor control and
sparing of normal tissue
Vascular damage
and angiogenic
response
Radiation induced
immuno-response