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Ultra high dose rate Synchrotron Microbeam Radiation Therapy.

Preclinical evidence in view of a clinical transfer

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We dedicate this paper to our friend and colleague Elke Bräuer-Krisch who has devoted 20 years of outstanding scientific research towards the clinical goals of MRT. None of the results reviewed in this paper could have been obtained without her contribution and perseverance. Elke passed away last year on September 10. We miss her.

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Summary (50 words)

This paper reviews the current state of the art of an emerging form of radiosurgery dedicated to brain tumor treatment and which operates at very high dose rate ($kGy.s^{-1}$). Microbeam Radiation Therapy uses synchrotron-generated X-Rays which triggered normal tissue sparing partially mediated by FLASH effect.

I-Introduction

Spatially fractionated radiotherapy was developed and reported in 1909 by Alban Köhler, a German radiologist, to reduce extensive damage to skin and adjacent tissues when he irradiated deep seated tumors with low voltage (60 – 70 kV) X rays. To this effect, he held a shielding iron wire grid tightly against the skin before exposure. The heavily irradiated, necrotic spots of the unshielded skin healed in few weeks. Variants of grid therapy were then used successfully in clinical external beam radiotherapy since the 1930's [1]. In the 1960s, Curtis, Zeman and colleagues at Brookhaven National Laboratory (BNL, USA) made an astonishing discovery during studies on cosmic radiation: Delivery of huge radiation doses (4000 Gy) by a microscopic, 25 μm thin, short deuteron pencil beam (20MeV) to the brain of mice did not damage blood vessels nor their normal brain tissue architecture, although nerve and glial cells died in the radiation path. Conversely, a similar, but 1 mm thick beamlet destroyed cerebral tissue and left a cavity behind [2]. Later, as a new synchrotron source (SLS) became available at BNL, D.N. Slatkin, who had witnessed those extraordinary results as summer student at BNL, decided to investigate the effects of synchrotron-generated X-ray microbeams on mouse brains with his colleagues [3]. The tissue lesions seen after those experiments resembled the lesions induced by the Curtis group [3,4]: no tissue necrosis developed in the brains of animals after focal administration of hundreds, even thousands of grays delivered along tissue microslices exposed to the peak doses of an array of regularly spaced microbeams (Fig.1). The dose microfractionation was maintained at large tissue depths; characteristic repair processes extended to deep tissues.

After a series of experiments at the SLS, on June 12th, 1992, a newly founded group for collaborative research on microbeams (P.Spanne, D.N. Slatkin, J. Laissue among others) presented the MRT concept to the ESRF Directorate (Professors Haensel, Altarelli and Brandén) in person, with a proposal for the construction of a beamline for preclinical experiments and clinical trials, including radiotherapy of cranial and spinal tumors in children. Thereupon, in September 1994, Spanne began working in Grenoble at the ESRF as a radiation physicist with the understanding that he was expected to develop MRT there too.

Three clinically important themes were investigated in preclinical animal experiments: Normal tissue tolerance to microbeams; MRT as radio-oncologic tool; microbeams for the treatment of non-malignant diseases such as epilepsy. For decades, accumulating preclinical biological data worldwide have supported the concept of a transfer of MRT from the laboratory to clinical applications. Work on hard- and software components to fit the safety criteria of a clinical trial was intensified, including the development of an image-guidance system, new detector systems for microdosimetry, new simulation approaches with mathematical modelling, continuous testing and adaptation of medical physics models to the synchrotron environment [5].

II-Preclinical translational research

Normal tissue radiotolerance

Adverse effects on normal brain tissues after radiotherapy of intracerebral tumors are main determinants of the prescribed radiation dose. Microbeam radiation therapy outstandingly preserves normal tissues from severe radiation damage which focuses on cells sited within the micrometer wide paths of microbeams, whereas cell death in tissue slices sited between the beamlets is minimal [3,4]. MRT also spares fast-growing immature tissues such as the duck brain in ovo [6] and in vivo, the cerebella of normal, suckling rat pups [7], adult rat and mouse brains [3,8–10], and of normal weanling piglets [11]. Normal tissues, even under development, are about 10 times more resistant to unidirectional microbeam irradiation than to conventional, broad beam (BB), exposure. Long term follow up demonstrated that normal tissues such as skin, cartilage, blood and lymphatic vessels are highly tolerant to MRT at entrance doses up to 400 Gy [12].

Microbeams can selectively ablate neurons, oligodendrocytes and astrocytes in the central nervous system without causing tissue necrosis. This surprisingly high radiotolerance of normal brain tissue relates to that of the normal vasculature of the brain and spinal cord. The close and very likely causal pathogenetic links between function, normal tissue damage and vascular alterations can be clearly visualized in adult rats in about one year after transverse irradiation of the cervical spinal cord by an ≈11mm long array of microbeams, *versus* irradiation by a 1.35mm wide single beam of similar X-rays. Microbeam peak / valley doses of 373.3 / 13.2 Gy produced foreleg paralysis in 50% of exposed rats; all rats exposed to peak/valley doses up to 253/9 Gy were paresis-free at 383 days post irradiation (dpi) [13]. Paresis developed around 50 to 60 days in all rats after spinal cord peak/valley doses of 507/18 Gy and 715/25.4 Gy. Of 10 rats exposed to peak/valley doses of 357/12.7 Gy, only two developed very late paresis. In rats exposed to a single beam the ED50 for paralysis was 130Gy, and no rat survived without paresis longer than 2–15 dpi after exposure to doses in the range of 454–146 Gy. The tissue architecture of the spinal cord was maintained in all rats exposed to microbeams (“MRT”), with minute (few square mm per cord) necrotic foci in the white matter after high peak doses (507 Gy and 715 Gy), often associated with fibrinoid necrosis of microvessels. Characteristic stripes also developed in the microbeam peak tracks. In sharp contrast, single beam irradiation with doses ≥182 Gy elicited early (2-15 dpi) white and gray matter necrosis that became wider than 1.35mm and progressed to liquefaction necrosis of the entire cervical spinal cord with increasing dose. Tissue necrosis was closely associated with focal hemorrhage, fibrinoid vascular changes and microvascular thrombi. In the brain of normal rats, no changes in morphometric parameters such as vessel size, density, permeability and blood volume were observed after unilateral exposure to microbeams (Fig.2). This phenomenon is responsible for the uninterrupted functional integrity – oxygen and nutrient supply - of brain microvasculature [10,14,15]. Further, MRT did not modify oxygenation of unidirectionally irradiated normal tissues [16]. After exposure of their cerebellum to an array of microbeams (highest peak and valley dose 262/12Gy), piglets remained developmentally, behaviorally, neurologically (no signs of paralysis) and radiologically normal [17]. ~~Cognitive impairment did not occur in irradiated animals (rodents and pigs) [17,18].~~ Mild, but transient cerebral oedema could be detected around one week after MRT in mouse brains [9,10]. The vascular tolerance is instrumental and is a major advantage of MRT even after very high peak doses (up to 1000Gy). However, normal peritumoral tissue can suffer cellular and vascular damage where multiple arrays from several irradiation ports cross [8,15] and the number of ports must be kept at a minimum.

Brain tumour control and vascular response

Irradiation by x-ray microbeam arrays were tested on different tumour models for preclinical cancer

radiotherapy. The relevance of irradiation geometry, use of crossfired arrays and very high radiation dose deposition for slowing growth and, in some cases, even curing 9L gliosarcoma implanted in rat brains was described in 1998 [8]. Although MRT has been applied to many other types of animal tumours (*e.g.* mammary carcinoma [19]), preclinical research has mainly focused on brain tumour models. Whatever the irradiation configuration used and the tumour treated, MRT proved to be significantly more efficient than conventional radiotherapy at equivalent doses (*i.e.*, when we consider the minimum valley dose that will cover the entire irradiation field as equivalent of the BB dose [20]); thus, unidirectional MRT significantly improved survival of rats bearing 9L intracranial gliosarcoma compared to BB; the efficacy of MRT and BB was similar when the MRT-dose was half that of BB [20]. ~~Moreover, aggressive intracerebral F98 tumours can be controlled by about 3 times lower equivalent MRT doses than a BB dose (unpublished data).~~ Tumour blood (and lymphatic) vessels might repair damage induced by very high radiation doses deposited by microbeams less effectively than normal mature blood vessels do. Microbeams have been shown to preferentially affect tumour vessels, significantly reduced blood volume, blood flow [15] and oxygenation [16], thus leading to tumour hypoxia [16] and tissue necrosis [15]. Vascular changes certainly contribute to MRT-induced tumour control, but other biological processes await identification. Since the immune system of zebrafish shows a high degree of overlap with the human system, caudal-fin regeneration model has been used to explore the effects of microbeams on responses of immune cells. Low doses of MRT temporarily destroy endothelia and thus denude the luminal blood vessel surface; attachment of thrombocytes and immune cells ensues [21]. Pangenomic analyses might drive preclinical research onto inflammatory response and cell cycle defaults but work is still needed to fully understand biological process underlying MRT efficiency [22,23]. Present preclinical evidence gathered worldwide may allow ~~us~~ to safely move to phase I/II trials at the ESRF, first on domestic animals, then in humans.

MRT as selective vascular disruptive agent

Preclinical work revealed that MRT slows the growth of tumours and sometimes ablates them. The underlying radio-biological mechanisms are only partially known. Could MRT serve as vascular disruptive agent, as an alternative to the rather disappointing current preclinical and clinical anti-angiogenic strategies for cancer treatment? Anti-angiogenic drugs have limitations: (i) Optimal intratumoral distribution of drugs requires elevated dose-levels which affect normal tissues [24] (ii) Reducing angiogenesis impairs the delivery of drugs, yields short-lived therapeutic gains and may promote malignant progression of tumours [25] (iii) those drugs do practically not work as single therapy and have adverse effects [26], a most disappointing drawback.

A model system that has contributed to the clarification of the question is the chicken chorioallantoic membrane. This almost pure vascular model with rapidly changing vasculature has an immature capillary meshwork that from day 8 to day 12 of development transforms into a hierarchical and mature microvascular system in which MRT exerts a “selective vascular toxicity” [27]. In this first embryonic model, MRT mediates its biological effects by selective disruption of immature capillaries that results in insufficient blood supply hours after treatment [28].

Conversely, mature capillaries covered by pericytes tolerate higher doses of MRT than their immature precursors. Using another model, we exposed regenerating zebrafish caudal-fins to synchrotron microbeams [21] to compare mature and newly formed, immature, blood vessels. The results confirmed that the vascular disruptive effects of MRT depends on the vascular maturation status in an adult organism. A chemotherapeutic agent was

injected intraperitoneally to 9L tumours bearing rats; JAI-51 coupled to MRT exposure led to an accumulation of the drug in the tumour (significant G2 cell cycle arrest) and a significant increase in median survival times whereas JAI-51 alone doesn't improve lifespan of animals [29]. The enhanced vessel permeability induced by MRT in brain tumours, demonstrated in Bouchet *et al.* 2010 [15] and later confirmed in Bouchet *et al*. 2017 [14] might be, at least in part, responsible for drug accumulation in 9L tumours.

III- Clinical Translation of MRT

Beamline status, patient alignment and Patient Safety System

The Biomedical Beamline (ID17) of the ESRF was initially devoted to the applications of synchrotron radiation to X-ray medical imaging. Pre-clinical MRT radiotherapy studies acquired imaging know-how that permitted to develop a new method for imaged-guided animal positioning and alignment for MRT of the brain. Radiographic imaging and a specially developed computer-interfaced system using the pink beam of the ID17 wiggler allowed precise irradiation field definition and radiation target positioning in animals with ~100-µm accuracy [30,31]. An automatic procedure now sets the beamline configuration either into imaging mode, *i.e.* low flux "pink beam" to achieve high quality X-ray images with doses not higher than 30 mGy, or into radiotherapy mode with the maximum photon flux. Thus, the total time spent by the animal/patient on the Patient Positioning System (PPS) does not exceed half an hour. The alignment method consists in combining standard scanner images and synchrotron images, both performed with stereotactic masks containing external fiducials. The measured positions of the fiducials are injected in an algorithm based on quaternions from which the PPS positions corresponding to the tumor centroid are extracted [32]. The main challenge to access to clinical trials with the microbeam synchrotron radiation is patient safety. A comparable radiotherapy modality has previously reached some clinical trial phase at ID17: Fifteen human patients have received "Stereotactic Synchrotron Radiation Therapy" (SSRT [33]). The PAtient Safety System (PASS) ensures delivery of the accurate irradiation dose within 2% tolerance. This system has been transferred, adapted to the MRT station (with a dose rate tolerance of 2%) and is now fully operational for future clinical trials.

Absolute dosimetry, TPS, quality insurance and required developments

High dose rate synchrotron radiotherapy programs towards clinical trials depend on collaboration of the clinicians, biologists, medical physicists and synchrotron scientists on the outcome of large animal trials; and on the degree of attainable accuracy in medical physics. Specific treatment planning [34], experimental dosimetry [35], patient positioning, safety and image guidance [32], for radiotherapy with beams as thin as 25 microns and for dose rates of few tens of kGy/s were developed.

Treatment planning programs for preclinical and clinical synchrotron radiotherapy have been extensively developed using Monte Carlo (MC) and hybrid calculation engines [36,37]. Absolute 1D experimental dosimetry using a microdiamond detector [35] as well as 2D and 3D microdosimetry techniques using radiochromic films [38,39] and polymers such as Presage® [40,41] are being worked out. Absolute dosimetry codes of practice using free air ion chambers for synchrotron [42,43] and electron FLASH radiotherapy [44], as well as biological dosimetry progress [45]. However, high dose rate radiotherapy programs are still in a conception phase. A specific patient positioning system must be built for clinical trials. The latter should fulfil constraints for MRT, such as: ≈0.1 mm positioning accuracy. Translational and rotational motors that enable irradiations from several ports;

a motor that ensures a vertical translation at constant speed for each irradiation, with a wobbling contained within a 10 micron envelope are needed. The current image guidance system [32], is time consuming and limited in precision: 1) the acquisition of two synchrotron beam radiographs and 2) the identification of markers on the thermoform mask; a system based on stereoradiography and registration on bone landmarks is the ultimate goal for MRT image guidance. The hybrid algorithm [34] is fast enough for patient dose calculation using two crossfired beams with ≈15% uncertainty for calculated minimum cumulated valley dose, compared to experimental measurements, but a higher precision is required such as in clinical radiotherapy/radiosurgery, as is benchmarking for clinical use (IAEA TRS 430 standards). Application of high dose rates and microbeam sizes, combined with complex irradiation multiport pattern, transcends theoretical limits of conventional dosimetry. Film dosimetry and high resolution gel dosimetry will be implemented, as well as transit dosimetry techniques based on diamond technologies for real time treatment monitoring.

Translating MRT to clinical application: schedule, design, first targets and dose prescription issue

This is a double challenge. A technological one: to ensure accurate positioning in the beam and to move the patient fast and precisely across it. Second challenge: for biologists, medical physicists and radiation oncologists to learn a completely new way to understand and prescribe the treatment dose. Current studies try to define more precisely the particular behavior of tumors and normal tissues facing MRT. For the first time in radiotherapy it seems that the homogeneous dose is no longer the main predictive measure for effects and adverse effects. First experiences led ~~us~~ to the acceptance of a dual aspect of the treatment: the valley dose for tolerance and toxicity, and the number of peak dose arrays for the cytostatic effect. ~~We~~ There are still ~~have to master~~ more effective ~~ely the~~ ways to be mastered to find ~~out~~ the relationship between peak dose in the array and the level of desired effects.

Beyond preclinical studies, the initiation of clinical trials will require step by step advances in a progressively increasing treatment complexity. As for previous SSRT studies with monochromatic synchrotron radiation, MRT will be, at first, used as a boost or a minor part of a standardized treatment, then progressively adapted to an integrated part of a standardized treatment. Intracranial targets of limited volume will be chosen thanks to the possibilities of very accurate immobilization and relatively small depth of the targets. Malignant or benign conditions requiring highly concentrated doses are considered. The present advance of large animal tests with MRT makes possible the application to humans as soon as ~~we have~~ the proper technical means to hold and move accurately a human patient will be available for MRT, hopefully in 3 to 5 years from now. MRT irradiations need to be fast to warrant a faultless spatial fractionation, executed in a single application per session, thus also ensuring a potential FLASH effect.

IV- MRT: was there a Flash Effect from the beginning?

MRT differs from conventional irradiation by spatial micro-fractionation and its extremely high dose rate (~ 16 kGy.s-1 versus 30 mGy.s-1). At such dose rates, biological effects are largely unknown. A recent study [46] suggests that high dose rate electron irradiations (≥40 $Gy.s^{-1}$) are well tolerated by normal mouse lungs, while differential effects between normal tissues and intrapulmonal tumours increased markedly, a phenomenon called FLASH effect": No fibrosis appeared then after doses below 20 Gy, whereas pulmonary fibrosis constantly appeared between 8 weeks to months after 15 Gy of conventional RT [46]. The "Flash effect" also manifested in

terms of apoptosis, preservation of blood capillaries and milder cutaneous lesions. High radiotolerance of normal tissues after electron FLASH RT was confirmed in higher mammals, e.g. in pig skin [47]. In a phase I veterinarian trial, single-dose electron FLASH escalation trial (25–41 Gy) 6 cat patients with locally advanced squamous cell carcinoma of the nasal planum showed an impressive progression-free survival of 84% 16 months after irradiation [47].

An increase in dose rate of total brain electron-beam irradiation reduced the toxicity to normal brain tissues [48]. The spatial memory was preserved two months after irradiation with dose rates exceeding 100 $Gy.s^{-1}$, whereas it was completely altered by a similar irradiation 10 Gy with a dose rate of 0.1 $Gy.s^{-1}$[48]. Recent results obtained at IMBL (Australian Synchrotron) highlighted the crucial role of very high dose rate, *i.e.* >100 $Gy.s^{-1}$. The authors did not observe any normal tissue sparing effect after irradiations performed at 37-41 $Gy.s^{-1}$ [49]. At very high dose rate, the "FLASH effect " could also be a factor component for the surprisingly high tolerance of normal brain tissues observed after X-ray MRT at the ESRF. A recent study provides a first proof for this concept [50]: A 10 Gy broad beam whole-brain irradiation of mice with synchrotron generated delivered X-rays at a mean dose-rate of about 17 kGy/s did not induce memory deficits up to 6 months after exposure. Correlates were preserved hippocampal cell division and reduced reactive astrogliosis (Figure 3). A comparable X-ray irradiation at conventional dose-rate (0.05 Gy/s, Pxi Precision X-Ray) irreversibly altered memory cognition [50]. Similar results were obtained in rats. Long terms studies (>18 months) to decipher dose-rate dependent adverse effects caused by conventional radiotherapy, synchrotron X-rays FLASH or MRT from those of spatial micro-fractionation are currently running. Neither synchrotron X-rays FLASH nor MRT panencephalic irradiations (10Gy) of healthy rats have induced motor deficiency up to 12 months after 10 Gy exposure (unpublished results). Several months after conventional irradiation in hospital mode the animals observed a new object in the same way as a familiar object, indicating memory deficit. Conversely, unirradiated animals, or those irradiated with synchrotron X-rays FLASH or with MRT, preferentially explored the novel object during the first six months after irradiation, which indicates preservation of memory. These first results link the preservation of cognitive functions to the use of high dose rates and suggest that FLASH effect may be a key factor for the tolerance normal brain tissues after MRT. The FLASH effect may thus pave the way for improved radiotherapeutic ratios for cancer treatment, MRT having shown significantly greater anti-tumoural therapeutic ratios than those of conventional broad beam irradiations.

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Figures

Figure 1 : A-Gafchromic film showing MRT irradiation pattern. Typical microbeams width used in preclinical experiments (rodents) are 25-50 µm wide, spaced from 200 to 400 µm apart. Peak to valley dose ratios calculated range from 15 to 40 in the literature. Dose profile is reported on B. C- pH2AX immunolabeling of DNA damage (red) induced by an orthogonal irradiation of the 9L gliosarcoma implanted in a rat's brain (Bouchet 2012).

Figure 2 : A-C Coronal section of a left parietal cortex, mouse brain, 1 year after irradiation with 25µm wide microbeams, 211µm on center. Skin entrance dose 1000 Gy (frozen sections, hematoxylin and eosin, low A) and high (B) magnification, extracted from [51]); C= Red immunolabeling of type IV collagen. D Estimated cortical blood volume (%) and number of vessels/mm²) 3 months after microbeam irradiation (312 or 1000 Gy) of the non-irradiated (control) and irradiated parietal cortex of nude mice. Mean values with their standard deviations are reported. Differences between the ipsilateral and contralateral hemispheres were not significant: two-tailed p values (paired t test) 0.05. Adapted from [51] and [10].

Figure 3: A- BrdU immunostaining on brain hippocampal sections performed two months post whole brain irradiation of mice with FLASH-X-rays (single dose of 10 Gy) or with X-rays delivered at conventional dose rate (CONV-X-rays). Control mice were sham-irradiated. Arrows point at BrdU positive clusters in the SGZ. Blue: DAPI; Red: BrdU. Clusters were counted in the whole hippocampal section. Statistical analysis performed with unpaired non-parametric Mann-Whitney test. *: $p < 0.05$ vs. control; **: $p < 0.01$ vs. control. Adapted from Montay-Gruel et al. 2018 [50].

B- GFAP immunostaining in brain striatum irradiated with FLASH-X-rays or with CONV-X-rays as in A; control mice were sham-irradiated: Reactive astrogliosis two months post irradiation. Arrows point at GFAP positive cells in the striatum. Immunoreactive cells were counted in striatum sections with MoreHisto software. Statistical analysis was done as in A. Adapted from Montay-Gruel et al. 2018 [50].

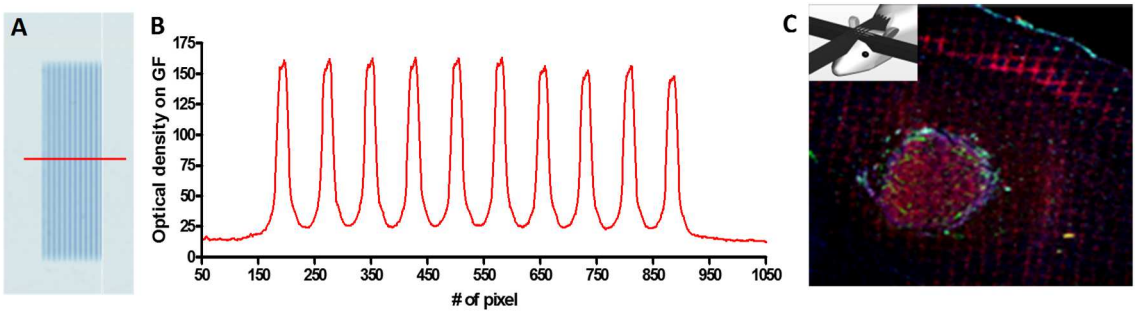

A
B
Optical density on GF
175
150
125
100
75
50
25
0
50
150
250
350
450
550
650
750
850
950
1050
# of pixel
C

Figure 1

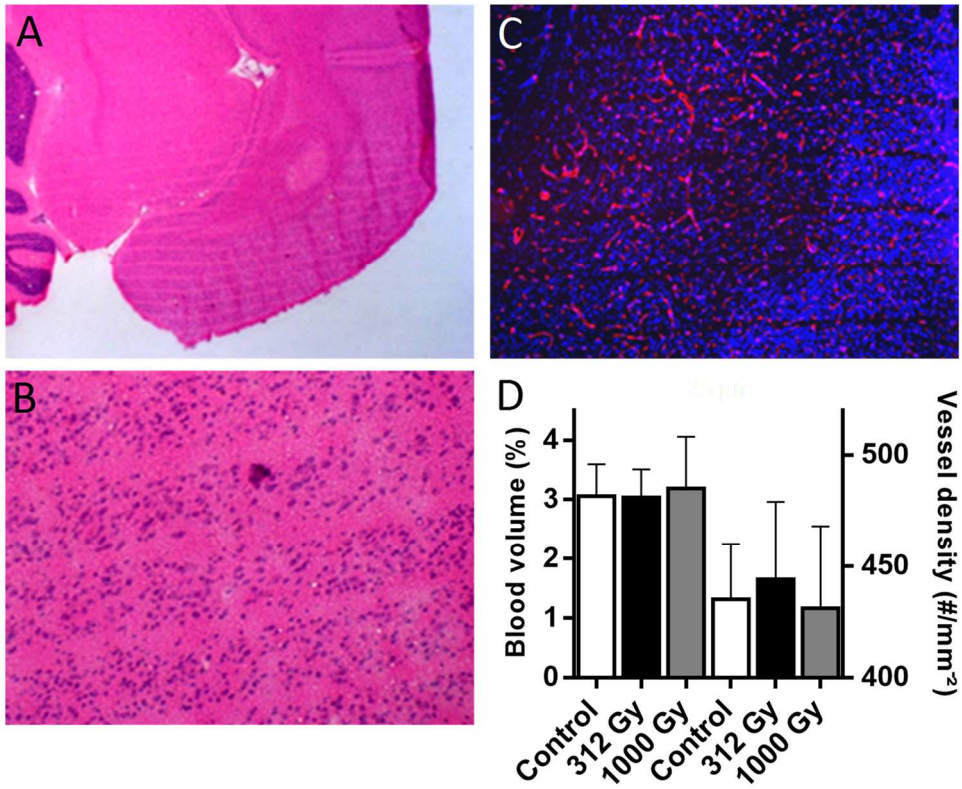

A
C
B
D
Blood volume (%)
Vessel density (#/mm²)
0
1
2
3
4
400
450
500
Control
312 Gy
1000 Gy
Control
312 Gy
1000 Gy

Figure 2

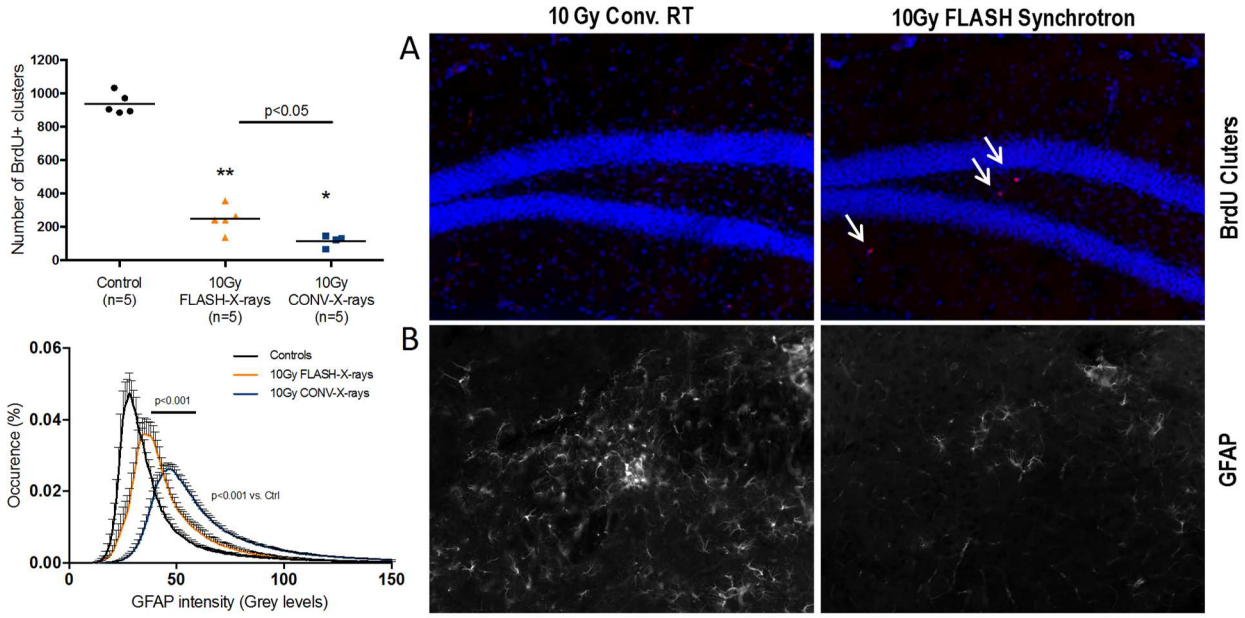

10 Gy Conv. RT
10Gy FLASH Synchrotron
A
B
BrdU Cluters
GFAP
Number of BrdU+ clusters
1200
1000
800
600
400
200
0
p<0.05
**
*
Control (n=5)
10Gy FLASH-X-rays (n=5)
10Gy CONV-X-rays (n=5)
Occurence (%)
0.06
0.04
0.02
0.00
Controls
10Gy FLASH-X-rays
10Gy CONV-X-rays
p<0.001
p<0.001 vs. Ctrl
0
50
100
150
GFAP intensity (Grey levels)

Figure 3