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RESEARCH ARTICLE

Feasibility Study on the nIORT® Adjuvant Treatment of Glioblastoma Multiforme through the Irradiation Field of Fast Neutrons Produced by a Compact Generator

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ABSTRACT

The glioblastoma multiforme (GBM) is the most malignant glial brain tumour with average survival time of 6÷18 months. Emerging evidence suggests that GBM cells appears to reprogram their tumour microenvironment, which is a highly heterogeneous and complex system, so that an efficient GBM radiotherapy (RT) should cover both the cells of the GBM and those of its microenvironment. Relying on a 5-year collaborative research study on the intra-operative radiotherapy (IORT) with fast neutrons - the so-called neutron-IORT (nIORT®) technique - the authors think that this objective could be achieved by using an ionizing radiation field of fast neutrons that behave in the biological tissues as a "foam field" hitting both the GBM cancer cells and the neighbouring microenvironment.

The nIORT® research activities - conducted by TheranostiCentre Srl, Berkion Technology LLC and ENEA - led to the fabrication of the first prototype of a compact neutron generator (CNG) that, through the deuterium-deuterium fusion reaction, produces neutrons of 2.45 MeV energy having: i) high linear energy transfer; ii) very high relative biological effectiveness (RBE), about 16 times higher than X-rays (and electrons) used in standard RT and IORT treatments; iii) reduced oxygen enhancement ratio, and hence resulting be very effective in cancer cells necrosis and apoptosis. The CNG is self-shielded, limited in size and weight (~120 kg) and manageable remotely by a robotic arm. A new prototype equipped by a cylindrical applicator to be inserted in the surgical cavities is currently under construction, with some technical advancements making possible its installation in an operating room dedicated to nIORT® treatments without posing any safety and environmental concern.

In this article the nIORT® potentiality was investigated in the view of the GBM treatment, but the study is however generalisable for the neutron irradiation of other brain cancer pathologies. Accurate Monte Carlo simulations, modelling the CNG equipped with a couple of cylindrical applicators of 4 and 6 cm in diameter inserted in the brain surgical cavity after craniotomy, demonstrated that the nIORT® device operated at 100 kV-10 mA DC supplies a neutron flux ~10^8 cm^{-2} s^{-1} and can deliver equivalent dose rates ~5 Gy (RBE)/min in the centre of the tumour bed. Thus, it could administer the clinical endpoints foreseen by the standard IORT protocols (~10-20 Gy (RBE)) in treatment times of few minutes, by providing a sort of "switching on and off neutron brachytherapy tool" without using needles of radioisotopes (e.g., ^{252}Cf). The near isotropic neutron emission allows to irradiate the tumour bed margins, normally filled by potential quiescent cancer cells, with lower (but still significant) dose levels. This should improve the local control of the tumour through the reduction of local recurrences and metastasis in the tumour microenvironment, and at the same time to avoid adverse effects of the administered neutron radiation field on the surrounding brain central nervous system. Also, the rapid decrease in tissue depth of the dose gradient (within few centimetres) should avoid any adverse effect on normal brain tissues and the neighbouring organs.

Nomenclature

CNG Compact neutron generator
DD Deuterium-deuterium
DSB Double strand breaks
EBRT External beam radiotherapy
EMT Epithelial–mesenchymal transformation
GBM Glioblastoma multiforme
GSC Glioma stem cell
HDPE High density polyethylene
HR Homologous recombination
ICI Immune-checkpoints inhibitor
IORT Intraoperative radiation therapy
IOERT Electron IORT
IOHDR High-dose rate brachytherapy
IR Ionizing radiation
LET Linear energy transfer
LEX-IORT Low energy X-rays IORT
LTC Local tumour control
MCNP Monte Carlo N-particle
NBT Neutron brachytherapy tool
NHEJ Nonhomologous end joining
nIORT Neutron IORT
OAR Organ at risk
OR Operating room
PE Polyethylene
QCC Quiescent cancer cell
RIAE Radiation-induced abscopal effect
RIBE Radiation-induced bystander effect
RISM Radiation-induced secondary malignancy
RT Radiation therapy
SSB Single strand break
TME Tumour microenvironment
TMZ Temozolomide
TT Treatment time

Symbols

$D'_{f,(n,g)}$ -Physical dose rate due to neutrons or photons (n,g) [Gy min^{-1}]
$D'_{eq,(n,g)}$ -Equivalent dose rate due to neutrons or photons (n,g) [Gy (RBE) min^{-1}]
$D'_{eq,tot}$ -Equivalent dose rate due to neutrons and photons (n+g) [Gy (RBE) min^{-1}]
$D_{eq,tot}$ -Equivalent dose due to neutrons and photons (n+g) [Gy (RBE)]
F_n -Neutron flux [cm^{-2} s^{-1}]
F_g -Photon flux [cm^{-2} s^{-1}]
Min -minute(s)

Introduction

The glioblastoma multiforme (GBM) represents the most aggressive and most lethal primary brain cancer with an average survival time of 6÷18 months. This neoplastic tissue grows slowly, local recurrence is the major cause for clinical deterioration (and deaths) and is frequently observed within 2÷3 cm from the initial lesion. The standard treatment for GBMs is the surgical resection followed by chemoradiotherapy, but the robust DNA repair and self-renewing capabilities of glioblastoma (and glioma) initiating cells promote resistance against this treatment modality. Thus, durable GBM management requires adjuvant radiation therapies, that are universally accepted as standard protocols in the areas of modern neuro-oncology.[1]

The radiation therapy (RT) treatment after the maximal safe surgical resection of the brain tumours is nowadays common in guideline recommendations. RT causes the death of tumour cells in target tissues by inducing DNA damages - as abasic sites, single-strand break (SSB) and double-strand breaks (DSBs) - either directly by the ionization track of the incident radiation or indirectly by oxidative stress phenomena generating reactive oxygen species (ROS) as free radicals, also affecting the tumour immune response. The irradiation aims to maximize the local tumour control (LTC) and hence lead to some benefits in overall survival and progression-free survival times of patients affected by high-grade brain tumours and other intracranial malignancies. The main debates remain with respect to the modality of radiation delivery: the dose target (to be administered in one-shot irradiation or by a fractionation schedule) and the time to initiation. With advancements in delivery techniques, over the past decades the intra-operative radiotherapy (IORT) has emerged as a very promising option with several studies demonstrating both feasibility and outcome equivalence, if not superiority when applied in the optimal setting, respect to the external beam radiotherapy (EBRT) for the treatment of primary and metastatic brain tumours.[2]

Since GBM and other brain cancers recur locally with a mean doubling time of the residual mass of few weeks (~3), any delay in starting adjuvant therapies results in significant re-growth of the tumour, making subsequent therapies incapable of lowering the residual burden below the "threshold" under which the LTC could be achieved by the immune system. Therefore, while the time to initiation for EBRT correlates with overall survival, the IORT seems to represent the most suitable treatment for patients with complete and incomplete resections in progressive glioblastoma. Indeed, IORT can be delivered as a single dose of radiation within the same anaesthesia episode of care in which the tumour is resected or biopsied and can allow to the tumour cells depletion (or at least a growth arrest of non-depleted cells) between surgery and adjuvant therapies (as chemotherapy and/or EBRT)[3].

Generally, the IORT techniques for the treatment of solid cancers exploit as ionizing radiation (IR) particles low-energy (~50 keV) X-rays (with the so-called LEX-IORT[4]) and high-energy (~5÷10 MeV) electrons[5,6] (IOERT). The most suitable technique for small target volumes, as sometimes occurs in recurrent inoperable GBM, is the high-dose rate brachytherapy (IOHDR) relying on a sealed radionuclide source being placed within the tumour resection cavity (e.g., needles of Californium-252[7]). The IOERT has been demonstrated to be feasible and effective in LTC of disease in breast, pancreas, soft tissue sarcomas, head and neck, uterine, and colorectal cancers: even if structural limitations of the cylindrical applicator tube have restricted use to cavities with clear line-of-sight parameters, some experiences describing an intracranial use in high-grade glioma were published.[8] Also the LEX-IORT devices with small applicators (such as Intrabeam®, Carl Zeiss Meditec AG, Jena, Germany) allow for a conformal apposition to the brain resection cavity walls.[3] They shown significant potential in recent clinical trials for GMB treatments and for a more widespread use in surgically resected intracranial metastatic disease.[9] It can be mentioned the Intraoperative Radiotherapy in Newly Diagnosed Glioblastoma (INTRAGO) I and II trials, in which the LEX-IORT (with a median administered dose of 30 Gy) was used as a boost for successive EBRT treatments (60 Gy) obtaining significant benefits for the LTC in patients with newly diagnosed GBM.[10]

Despite the prospective outcome data are still limited, the IORT advantages - due to the instant prevention of tumour regrowth, optimised dose-sparing of adjacent healthy brain tissue and immediate completion of metastases treatment - are nowadays considered comparable with the long-term outcomes of adjuvant EBRT.[11] But, being the GMB a highly heterogeneous and dynamically complex tumour that usually grows back within 6÷9 months of initial diagnosis and treatment, its cancer cells can survive to the standard RT treatments by changing or adapting to their irradiated tumour microenvironment (TME).[12,13] Experimental studies show a significant difference between the radiosensitivity of GBM cells *in vitro* versus *in vivo* that can be attributed to the TME[14]. Indeed, as the GBM grows, it spreads into the surrounding brain tissues and several mutations happen in genes within the tumour cells themselves, as well as in their TME. A peculiarity of the GBM is to slip "on-switches" different genes belonging to its "DNA-circles" to drive tumour grow and alter the normal biological mechanisms of cell cycles arrest and apoptosis.

To induce necrosis and apoptosis of the GBM cancer cells cycles it is necessary to administer high dose levels in a limited treatment time (i.e., high dose rate) immediately after the surgical rejection of the primary neoplastic masses, as foreseen by the IORT modality in which (differently from EBRT) the radiation damage in normal tissue is limited by craniotomy. In fact, while low dose levels trigger apoptosis of cancer cells but are unable to induce an effective antitumor response, high doses promote necrosis of cancer cells and are more effective in triggering both innate and adaptive antitumor responses. Additionally, while low doses induce biological effects as inflammatory reactions, innate immune activations and DNA repairs (as adaptive response), preclinical studies showed that high doses per fraction, e.g., > 8–10 Gy (RBE), are more effective in increasing the antitumor response.[15]

To reach high dose rates, besides the flux level of IR particles in the tumour bed (proportional to the beam power), the type of IR used represents a key factor. Particles with high linear energy transfer (LET) and high relative biological effectiveness (RBE) in the irradiated tissue should induce the necrosis of the GBM cancer cells cycles in the shortest possible time compared to their repopulation and redistribution times. The cells damage is more dispersed and uniform with low-LET IR (as X- or γ-rays) and more discrete, clustered, and heterogeneously scattered along the beam tracks with high-LET particles.[17] For this reason, the authors believe that the adjuvant IORT adopting the fast neutrons as IR particles – the so-called nIORT® technique[16], amply described and discussed in this article - could be the best option in terms of median survival for the treatment of the GBM, hopefully even without adjuvant administration of chemo-therapeutic agents as temozolomide (TMZ). Indeed, the 2.45 MeV energy of the IR beam - produced by a compact neutron generator (CNG) through the deuterium-deuterium (DD) fusion reaction - allows to a high LET and to a very high RBE: about 16 times higher than the photons and electrons (RBE unitary) value. Furthermore, the almost-isotropic spatial distribution of the IR beam permits to irradiate large target area including the tumour bed margins and the TME: thus, the nIORT® could potentially represent a therapeutic option for the LTC aiming the "near-total" disappearance of metastases by avoiding any local recurrence after the treatment.

It is worth to notice that this research study deals only with the radiological aspects associated with the nIORT® irradiation of head tissues and does not deal with any other relevant clinical issues related to the specific brain cancer pathology. The

feasibility study has been carried out by simulating - with accurate Monte Carlo code calculations - the CNG equipped with the typical IORT cylindrical applicators (4 and 6 cm in diameter) inserted in the brain surgical cavity. The potential advantages deriving from the high dose rates – up to 5 Gy (RBE) per minute (min) in the centre of the tumour bed – and from the "physical/biological" peculiarities of the fast neutrons IR are discussed in the following sections, together with a brief description of the CNG conceptual design. Differently from the Boron Neutron Capture Therapy (BNCT) which uses thermal and epithermal neutrons to induce (n, α) reactions in boron carriers injected into the patient[18,19], the nIORT® technique employs fast neutrons that interact directly and efficiently with the hydrogen nuclei by producing recoil protons that ionize the tissues. Thus, giving-up the boron selectivity, the tumour bed is directly irradiated with fast neutrons exploiting their high LET and very high RBE values.

Radiation beam features in standard IORT techniques

The choice of the most eligible IORT treatment, as adjuvant radiation therapy for brain metastases after surgical rejection, is strictly related with the tumour bed (and patient) conditions. Depending on the volume, location and sensitivity of the tumour and the surrounding normal tissue, different techniques may be selected. For a proper comparison among the main figures of merit of the different options and the subsequent choice of the most eligible treatment, the physical features of the IR particles adopted - and their biological effects in the irradiated tissues – must be considered at first. Besides the dose (rate) level, the following parameters can be used to identify the main physical and "biological" features of the IR beam:

a) the LET and RBE values of the particles;
b) the dose gradients in tissues depth;
c) the beam area and the dose homogeneity on superficial tissues.

In the choice of the most suitable IORT treatment, the IR beam features (a÷c) must be properly considered in dependence of the tumour bed superficial extension and depth.

The low-LET IR (as X-rays) induces simple DNA lesions that are quite efficiently repaired by cells, whereas high-LET radiation causes complex DNA lesions difficult to repair and thus enhancing cancer cells killing.[17] About the RBE, that is 1 for photons (by definition[20]) and about 1 also for electrons[21], recent studies indicate that the LET of LEX-IORT produces more lethal macromolecular damage than IOERT.[22] The RBE value of the currently used 50 kV X-rays medical devices for LEX-IORT was measured in a phantom model in the range of 1.26 to 1.4.[23]

The dose gradients in tissues depth are strictly related with the IR physical properties (e.g., LET). In the IOERT, the dose distribution is peaked slightly below the surface tissues (on which the maximum dose is usually required in the IORT modality) and present a smooth gradient in depth with significant dose levels until 3÷5 cm (depending on electrons energy). The LEX-IORT exhibits a steeper dose gradient with most of the dose delivered within 0.5÷1 cm from the applicator surface. The smooth or steep dose gradient can be advantageous or detrimental depending on the treatment objectives. For LTC in metastatic tumour cavities, the steep gradients of LEX-IORT can provide maximal dose to non-visualized microscopic disease. On the contrary, rapid dose decrements can allow for under treatment of residual disease and in such case the IOERT could be beneficial (especially when it represents the only adjuvant radiotherapy after surgery[3]). Hence, the choice of the more appropriate IORT technique requires an appropriate balancing between the aimed LTC and, at the same time, the adverse effects of the radiation on normal tissue.

The homogeneity of dose delivered in the beam area also impact some outcomes and some evaluations are needed in the selecting process for the most eligible IORT modality. As an example, the dose administered via IOERT are more evenly distributed across a tissues area than those achieved by IOHDR.[24] Also LEX-IORT devices present homogeneity in dose distributions during single fraction treatments.[25] Finally, for what concerns the beam extension, it can be noticed that the treatment of relatively small target volumes can be achieved successfully with existing technologies, such as LEX-IORT and IOHDR.[3] On the contrary, relatively large tumour beds with significant topographic irregularities remains a therapeutic challenge even for the IOERT, having a focused beam most reliable for flat tissue surfaces. Residual metastatic diseases present at the margins of an extended tumour bed (and TME) cannot be irradiated effectively by focused electrons: the beam "brushing" should be adopted, that is impracticable in the brain cancers treatment with craniotomy (in which the IOERT applicator has to be inserted in the resected skull cavity).

Radiation beam features in nIORT®

To enhance the IORT potentiality for brain cancer treatments, fast neutrons can be used as IR particles with the nIORT® technique[16], invented by the TheranostiCentre Srl company (TC, Italy) and further developed in collaboration with the Berkion Technology LLC company (BT, USA) and the Italian

National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA). Besides the effectiveness of high LET / RBE neutrons in cancer cells killing, in principle the nIORT® could extend the IORT applicability beyond the "focused beam" IOERT and "small target volume" LEX-IORT and IOHDR techniques. Indeed, as here shown, the almost spatial isotropic irradiation field of the IR neutron beam is well suitable for irradiating tumour bed margins in irregular surfaces and acts as a sort of ionizing radiation "foam" filling the surgical cavity, thus allowing to kill potential quiescent cancer cells (QCCs) in the TME.

The research program on nIORT® is currently ongoing with the experimental tests of the first prototype of the CNG[26] designed, patented[27], developed and built by TC, BT and ENEA. The CNG irradiation performances for materials irradiations are going to be measured in a new equipped ENEA laboratory.[28] The schematic diagram on the left side of Fig. 1 summarizes its main design features consisting of three main components: the ion source (i.e., plasma chamber with D, a nonradioactive isotope of hydrogen), the acceleration column made of High-Density Polyethylene (HDPE) and the beam target electrode which is made of titanium. The positive deuterium ions (D+) created in the RF-driven source chamber are accelerated in the HDPE column to the titanium target where the DD fusion reaction occurs by generating neutrons of 2.45 MeV energy. The DD-CNG operates at 100 kV - 10 mA DC producing a neutron yield of 3.3 x 10^9 s^{-1} at the titanium target and a flux of ~10^8 cm^{-2} s^{-1} at the irradiation window close to the target[29,30].

The right side of Fig. 1 shows a picture of the HDPE accelerator column (about 15 cm in diameter, having excellent properties in shielding neutrons) and the RF plasma chamber attached to it.

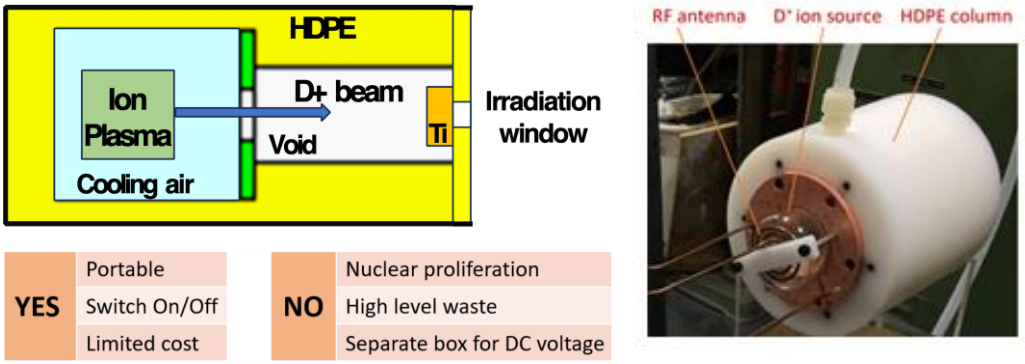

Fig. 1: Conceptual design and main features of the D+ ion-based CNG (left). Picture of the CNG accelerator column with the D+ ion source plasma chamber (right).

In parallel with the experimental characterisation of the first CNG prototype, a new one is currently under construction. It is built with some technical advancements – such as operation reliability, safety, and radiation protection aspects - that should make the CNG suitable to be installed in an operating room (OR) dedicated to nIORT® treatments, without posing any safety and environmental concern. Instead of having an irradiation window for materials irradiation purposes (as in Fig.1 for e.g., *in vitro* tests on commercial cancer cells), the new CNG prototype foresees a cylindrical applicator pipe - typical of IORT treatments - to be inserted in the surgical cavity.

Several studies with Monte Carlo codes have been performed to design the CNG shields and to optimise its architecture in the view of potential nIORT® irradiations. By referring the CNG design equipped with a 6-cm-diameter cylindrical applicator, Fig. 2 shows the computed 2D maps of the neutron flux distributions (in arbitrary units) inside the IORT applicator (marked by a thicker purple line) and in the biological tissues of the surrounding brain surgical cavity. By referring the three criteria (a÷c) previously defined to characterise the IR beam features, the following aspects can be remarked:

a) The high LET (~ 40 keV/mm as average[31]) and very high RBE (@16[20]) values of fast neutrons, that should result in very efficient killing of cancer cells. These IR features may be advantageous for treating radioresistant tumours that require superior dose conformity by reducing the integral dose and sparing surrounding healthy tissues and critical organs, minimizing treatment-related complications, and reducing the risk of radiation-induced secondary malignancies[17] (RISMs).
b) The dose peak released at the tissues surface and its rapid decreases in few centimetres in tissues depth (see left frame of Fig. 2 and Fig.

5), that should spare normal tissues and the neighbouring Organs at Risk (OARs).

c) The diffuse spatial dose distribution of the neutrons' beam on superficial tissues (see right frame of Fig. 2). Thus, relatively large tissue areas can be irradiated with quite-high uniform dose levels in the tumour bed centre and, aiming the LTC, with lower dose values administered in the tumour bed margins and surrounding normal tissues. Since IORT (and more generally RT) must balance delivering a sufficient dose to eradicate the tumour against limiting the real risk for adverse responses in normal tissue, also this beam peculiarity may be significantly advantageous.

In a previous work, the potential CNG performances were evaluated for the nIORT® irradiation of the breast cancers[32]. Here we have explored the potentiality for the GBM treatment by inserting IORT applicators of different diameters in the skull after craniotomy and positioning them via hard-docking close to - or in contact with - the brain tumour bed. The applicator diameters chosen in the Monte Carlo simulations - 4 and 6 cm - should be well suited for the GBM (and other brain cancer treatments) since, as mentioned, local recurrences are majorly observed within 2-3 cm from the initial lesion.

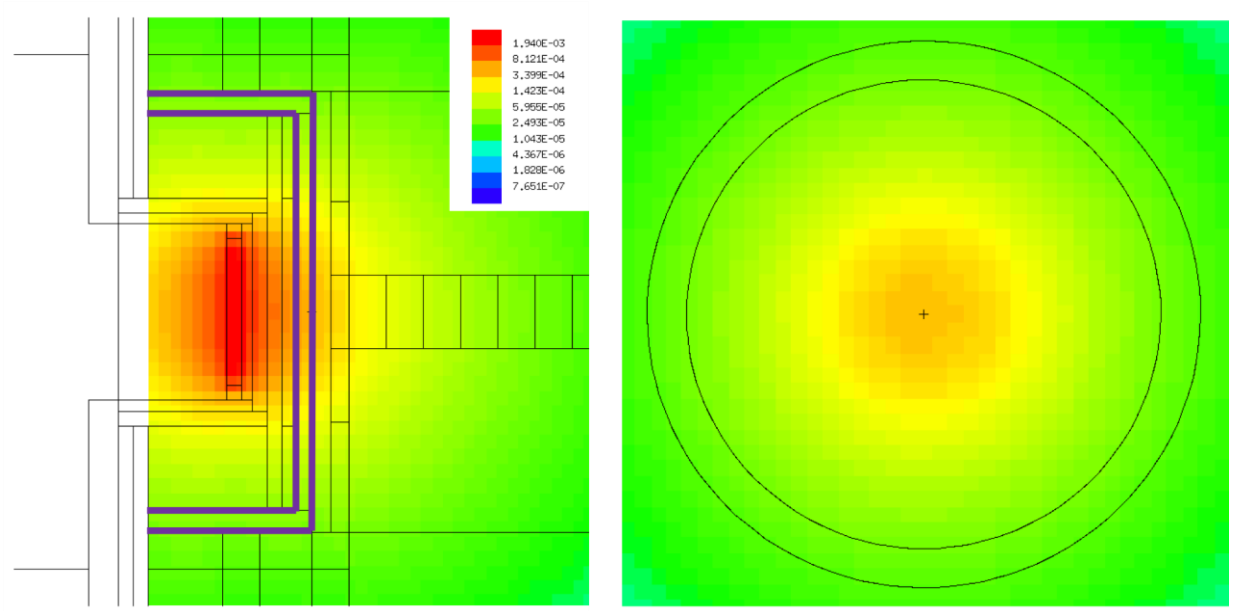

Fig. 2: 2D neutron flux spatial distributions (in arbitrary units) inside the 6-cm-diameter cylindrical nIORT® applicator and in the surrounding biological tissues of the brain surgical cavity after craniotomy. The 2D flux distributions in a plane along the symmetry axis of the applicator (marked by a thicker purple line; left) and in a plane parallel to the circular applicator end-cap (right) are shown.

Methodology

Accurate Monte Carlo simulations were carried out by means of the Monte Carlo N-Particle (MCNP) ver. 6.1 code[33] - coupled with the most up to date ENDF/B-VIII.0 nuclear data[34] - to evaluate the neutron flux (see Fig. 2), the physical (Gy) and equivalent (Gy (RBE)) dose distributions in biological tissues surrounding the IORT applicator. The neutron yield generated by the 100 kV-10 mA D^+ ions impinging on the titanium target - about 3.3 10^9 s^{-1} with an almost isotropic direction of emission – has been reproduced accurately with the MCNP code. The simulations are not restricted to neutrons (*i.e.*, the primary ones from the titanium target and the secondary ones coming from the scattering with CNG walls and biological tissues), but they also include the photons: *i.e.*, gammas (g) created by neutrons interaction with matter. The physical dose rates (Gy/min) due to neutrons ($D'_{f,n}$) and gammas ($D'_{f,g}$) were calculated in the tissues of the brain surgical cavity. The total equivalent dose rate ($D'_{eq,tot}$; Gy (RBE)/min) administered results to be:

$$D'_{eq,tot} = D'_{eq,\gamma} + \int w_R(E) D'_{f,n}(E) dE \quad \text{[Gy (RBE)/min]} \qquad (1)$$

where:

- "$w_R(E)$" is the radiation weighting factor[20,35] for neutrons (@16 at 2.45 MeV);
- the radiation weighting factors for photons is one (i.e., $D'_{eq,\gamma} \equiv D'_{f,\gamma}$);

- the level of the photon flux in superficial tissues is about twenty time lower than the neutron one. Because of higher flux (@20x), LET (@5x) and RBE (@16x), the neutrons contribution to the total dose rate (1) results three order of magnitude higher than the photons one.

The equivalent dose due to neutrons was calculated by the MCNP code starting from the flux and physical dose spectra in biological tissues and the corresponding weighting factor w_R, whose behaviour with energy[20] was accurately interpolated by a linear fit of 13 energy groups. Clearly, the RBE value obtained (≃16 in superficial tissues, as also computed by MCNP code) will have to be validated experimentally, e.g., by irradiating with the CNG-nIORT® device an anthropomorphic water phantom endowed with vials.

Starting from the equivalent dose rate results in the biological tissues, it is possible to estimate the Treatment Time (TT) needed to administer the aimed dose targets (defined by standard clinical protocols) and, at the same time, to evaluate the dose spatial distributions around the peak values in the centre of the brain tumour bed: tumour bed margins, skull and skin, whose compositions were retrieved from reliable MC human phantoms' models in literature.[36]

Simulation of nIORT® treatments

The left side of Fig. 3 shows a 2D section of the MCNP model of the CNG and surrounding shields, made of borated PE and an external layer of lead (mainly for g rays). The whole system is a cylinder with about 30 cm in diameter and 40 cm in length. For simplicity, the MCNP model does not consider the ion source chamber (in the back part of the acceleration column, see Fig. 1) since the simulations start from the (near isotropic) spatial distribution of the 2.45 MeV neutrons emitted from the titanium target on the opposite side of the CNG: thus, this model simplification has no impact on the flux and dose rate results into the biological tissues.

The right side of Fig. 3 shows an enlarged drawing of the MCNP model of the cylindrical IORT applicator (in purple colour), shaped around a HDPE bearing-ledge structure which contains an aluminium holder for the titanium target. The applicator pipe made of Lucite ($C_5O_2H_8$) is almost transparent for neutrons and, via hard-docking, can be inserted close to (or in contact with) the tumour bed inside the surgical cavity. For brain cancers irradiation, the IORT applicator is positioned in the skull "opening" surrounded by skin and skull tissues. The skull was modelled 0.7 cm thick[36] and its covering skin 0.5 cm thick (> 0.3 cm[36] to consider "folds"). Obviously, the tumour bed extension is not so well-delimited as in the MCNP model (with a net separation between the brain tumour bed and normal tissues, as in Fig. 3) but, in any case, the flux and dose rate levels were accurately calculated / monitored in small volumes for all the tissues around the IORT applicator.

The right side of Fig. 3 refers to an IORT applicator of 6 cm diameter and about 2 cm long: the air gap between the CNG walls and the patient's head skin results about 1 cm. A thin air gap (0.25 cm thick, light blue cell) occurs between the applicator end-cap and the tumour bed, that is to consider an average distance between them for the "not uniform" contact due to tissue irregularities.

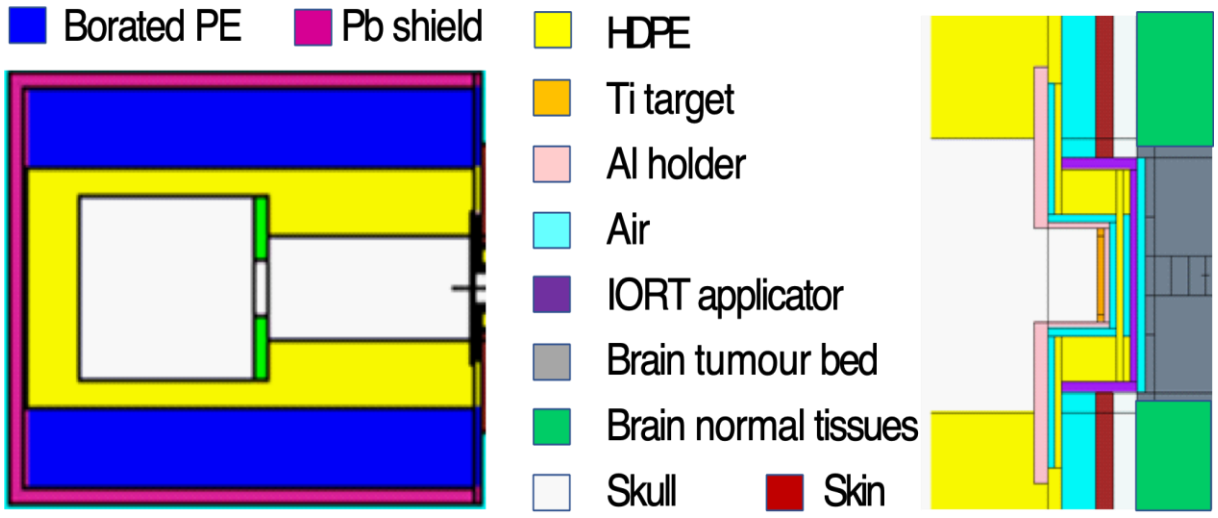

Fig. 3: Vertical section of the MCNP model of the CNG and surrounding shields (left). Zoom section of the MCNP model of the 6-cm-diameter cylindrical IORT applicator and surrounding head biological tissues (simulating the nIORT® treatment of brain, right).

Results with 6-cm-diameter nIORT® applicator

As shown in the left side of Fig 4, the surface tissues of the surgical cavity were modelled in MCNP with 2.5 mm thick cells (of cylindrical shell shape, numbered in red) around the IORT applicator. Besides the air gaps in light blue, the brain tumour bed is represented by the dark grey cells (301, 302, 303, 304), the skull by light grey cells (305) and the skin by dark red cells (302). The graph in the right frame of Fig. 4 reports the dose rate values obtained by MCNP in these superficial cells.

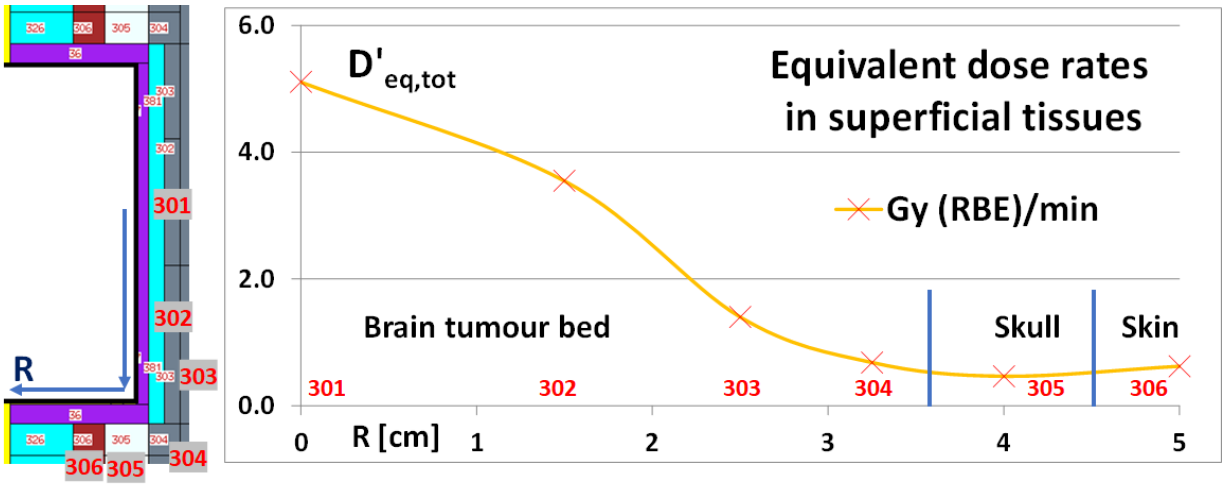

Fig. 4: Zoom section of the MCNP model of the 6-cm-diameter nIORT® cylindrical applicator inserted in the brain surgical cavity (left: red numbers refer to MCNP cells numbering). MCNP results for equivalent dose rates in surface tissues in front and around the applicator (right).

To be observed the @ 5 Gy (RBE)/min dose rate peak reached in the centre (cell 301) and the quite high values in the rest of the brain tumour bed (cells 302, 303), together with the decisively lower values in the tumour bed margins (cell 304), skull and skin tissues (cells 305, 306). To be noticed that, as sketched in the left part of Fig. 4, the "R" horizontal axis in the dose rates' graph represents the radius of the tumour bed tissues in correspondence of the applicator end-cap until 3 cm, while bigger R values refer to the skull and skin tissue on its lateral side.

Despite the almost isotropic neutron field, the greatest part of the dose is administered in the forward direction, while the tissues on the lateral side of the applicator (tumour bed margins, skull, and skin) receive dose levels decisively lower (9÷12% of the peak). As reported in Table 1, while the peak dose rate reaches 5 Gy (RBE)/min, the average value in the surface tissues of the whole tumour bed (modelled with 6 cm diameter) results of about 1.4 Gy(RBE)/min and the minimum is limited to 0.7 Gy(RBE)/min at the bed margins (slightly greater than values in skull and skin). As a Monte Carlo code, MCNP is affected by the statistical noise of the results due to its stochastic nature. For easiness, the uncertainty of the dose rate results is not indicated: their relative standard deviation is however lower than ~1%.

Table 1:MCNP results for equivalent dose rates in surface tissues of the brain surgical cavity in front and around the cylindrical nIORT® applicator (6 cm diameter).

Dose Rate	Tumour Bed			Skull	Skin
	Peak	Average	Minimum	Peak	Peak
Gy (RBE)/min	5.1	1.4	0.7	0.5	0.6

Fig. 5 shows the equivalent dose rate profiles in brain depth: the dose rates were sampled with 0.25÷0.5 cm thick cells (1 cm in radius) along the symmetry axis of the applicator. It can be clearly seen that the dose level drops rapidly decreasing by a factor 2 at ~1 cm depth and by a factor 5 at ~2.5 cm depth. Hence, the overwhelming part of the dose is administered in the surface tissues of the surgical cavity and quite high doses are released until about 1 cm depth, while in deeper tissues it strongly decreases avoiding an "excessive" irradiation of normal brain tissues and closest OARs.
By comparing the dose gradient in Fig. 5 with the flux distributions in the left frame of Fig. 2, it can be deduced that the neutrons diffuse into tissues but, because of their high LET, the overwhelming part of the dose is released at surface and in the first 1÷2 cm depth. The deep tissues are still irradiated by

the thermal and epithermal tails of the neutron flux having decisively lower LET and RBE values, and hence significantly less effective in cells damaging.

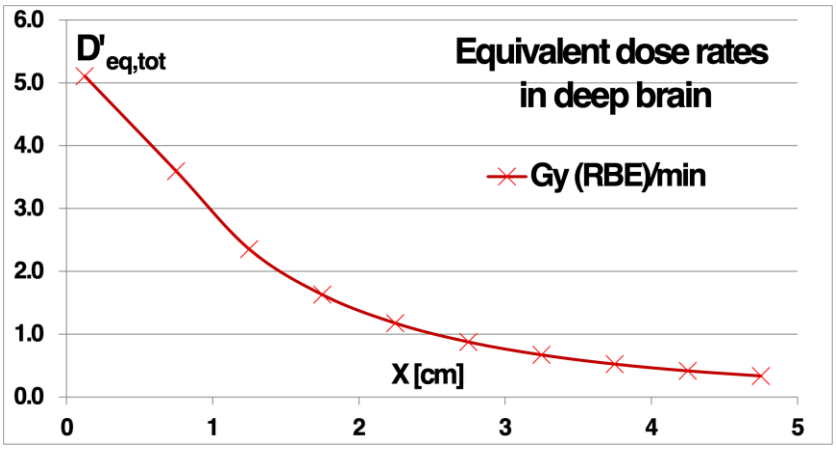

Fig. 5: MCNP results for equivalent dose rate profiles in brain depth (6-cm-diameter cylindrical nIORT® applicator).

Results with 4-cm-diameter nIORT® applicator

The left side of Fig 6 shows the MCNP model of the 4-cm-diameter cylindrical applicator and the surrounding brain surgical cavity. As in the 6-cm-diameter case, the surface tissues were modelled with 2.5 mm thick cells of cylindrical shell shape. The graph in the right side of Fig. 6 shows the dose rate values obtained in these superficial cells: the "R" horizontal axis represents the radius of the tissues in correspondence of the applicator end-cap until 2 cm, while bigger R values refer to the skull and skin tissue on its lateral side (similarly to Fig. 4).

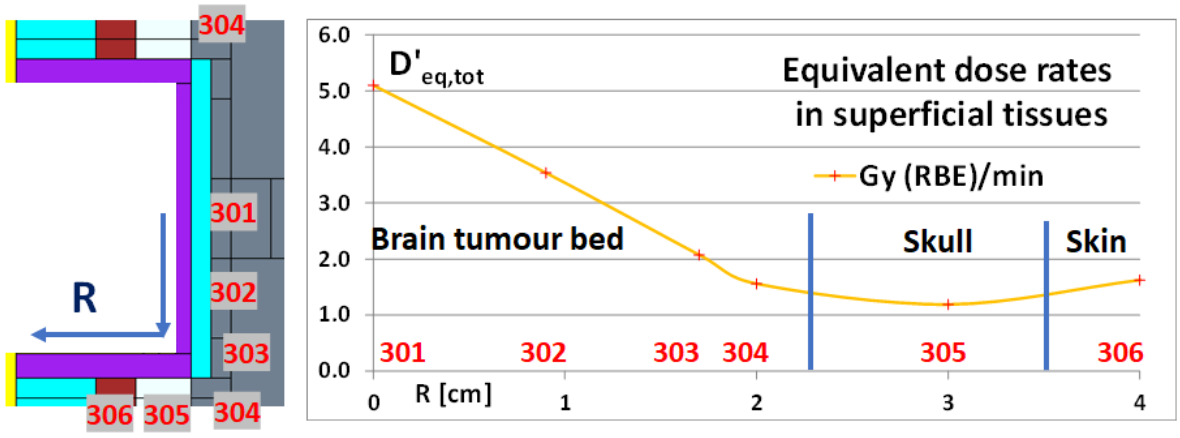

Fig. 6: Zoom section of the MCNP model of the 4-cm-diameter nIORT® cylindrical applicator inserted in the brain surgical cavity (left: red numbers refer to MCNP cells numbering). MCNP results for equivalent dose rates in surface tissues in front and around the applicator (right).

As reported in Table 2, the dose rate peak in the centre of the tumour bed is still ~5 Gy (RBE) / min (as in the 6-cm-diameter case), while the maximum dose rate levels in skull and skin result about 20% and 30% of the peak. Therefore, with the 4-cm-diameter cylindrical applicator, the greatest part of the dose is also administered in the forward direction, but with:

- 2.5 Gy(RBE)/min as average dose rate value in the surface tissues of the whole tumour bed (vs. 1.4 Gy(RBE)/min with 6 cm diameter);
- 1.2 and 1.6 Gy(RBE)/min as maximum dose rate values in skull and skin tissue on the lateral side of the applicator, respectively (i.e., 20-30% of the peak value vs. 9-12% with 6 cm diameter).

Table 2: MCNP results for equivalent dose rates in surface tissues of the brain surgical cavity in front and around the cylindrical nIORT® applicator (4 cm diameter).

Dose Rate	Tumour Bed			Skull	Skin
	Peak	Average	Minimum	Peak	Peak
Gy (RBE)/min	5.1	2.5	1.6	1.2	1.6

Finally, Fig. 7 shows the equivalent dose rate profiles in brain depth: the dose rates were sampled with 0.25÷0.5 cm thick cells (with 1 cm radius) along the symmetry axis of the applicator. As in the 6-cm-diameter case (Fig. 5), the dose level drops rapidly decreasing by a factor 5 at ~2.5 cm depth.

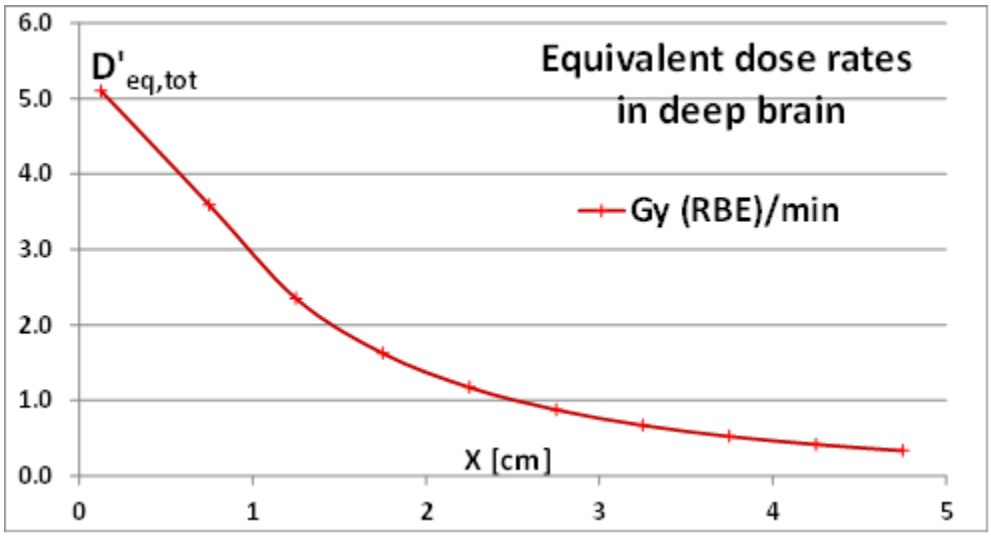

Fig. 7: MCNP results for equivalent dose rate profiles in brain depth (4-cm-diameter cylindrical nIORT® applicator).

The CNG performances for potential nIORT® treatment plannings

In view of possible clinical applications, the CNG performances for potential nIORT® treatments were evaluated by:

- adopting the clinical endpoints defined in the standard IORT techniques[37];
- deducing the correspondent TT needed to administer such dose targets in a single session from the equivalent dose rate results (1).

Referring to the IOERT, in dependence of the tumour bed (and patient) conditions, two dose targets are conventionally adopted: the so-called Boost IORT and Radical IORT regimes foreseeing clinical endpoints of about 10-12 and 20 Gy(RBE), respectively.[38]

Tables 3 and 4 show the irradiation performances in different dose regimes of the CNG equipped with IORT applicators of 6 and 4 cm in diameter, respectively. The 10 and 20 Gy(RBE) levels were assumed as dose targets for the Boost and Radical IORT regimes that, thanks to the high dose rates, can be administered in TTs of few minutes. Even much higher dose targets - in the so called Ultra-Radical regimes, up to 50/75 Gy(RBE) in Tables 3 and 4 - could be administered in limited TTs, as sometimes it should be required in the most severe high-glioma and GBM cases.

In some details, in the Boost and Radical IORT regimes - foreseeing 10 and 20 Gy(RBE) as peak dose target in the tumour bed - the TT is limited to about 2 to 4 minutes with both IORT adaptors. Otherwise, by referring to the average dose targets in the whole tumour bed (modelled with a diameter of 6 or 4 cm in correspondence of the chosen adaptor):

- with 4 cm diameter (Table 4), by delivering 10/20Gy (RBE) as average dose in about 4/8 minutes, the peak dose administered in the tumour bed centre reaches up to ~ 20/41 Gy(RBE).
- with 6 cm diameter (Table 3), by delivering 10/20 Gy(RBE) as average dose in about 7/14 minutes, the peak dose administered in the tumour bed centre reaches up to ~ 36 /71 Gy(RBE).

Trivially, the lower diameter leads to an increase of the average dose in the whole tumour bed and of the minimum dose in the bed margins. Actually, some of the latter cases can be classified as belonging to the Ultra-Radical IORT regime, as well as the last two results reported in Tables 3 and 4 having 50 / 75 Gy(RBE) as peak dose target in the tumour bed

centre, that can be reached with both adaptors by a single irradiation spot of about 10 / 15 min only.

It can be noticed that in the BNCT field the 12.6 Gy (RBE) limit is usually assumed as peak dose for the healthy tissues.[39] Tables 3 and 4 indicates that this limit is exceeded in the skull and skin tissues only with the IORT applicator of 4 cm diameter in the 50÷75 Gy(RBE) Ultra-Radical dose regimes.

Table 3: CNG irradiation performances for potential nIORT® treatments in Boost (10 Gy(RBE)), Radical (20 Gy(RBE)) and Ultra-Radical (> 20 Gy(RBE)) regimes (6-cm-diameter cylindrical applicator).

Dose target	TT	Gy (RBE)				
		Tumour Bed			Skull	Skin
Gy (RBE)	Min	Peak	Average	Minimum	Peak	Peak
10 as Peak	**2.0**	**10**	2.8	1.3	0.9	1.2
10 as Average	**7.0**	35.6	**10**	4.7	3.2	4.3
20 as Peak	**3.9**	**20**	5.6	2.7	1.8	2.4
20 as Average	**13.9**	71.2	**20**	9.5	6.5	8.7
50 as Peak	**9.8**	**50**	14.0	6.7	4.5	6.1
75 as Peak	**14.7**	**75**	21.1	10.0	6.8	9.1

Table 4: CNG irradiation performances for potential nIORT® treatments in Boost (10 Gy(RBE)), Radical (20 Gy (RBE)) and Ultra-Radical (> 20 Gy (RBE)) regimes (4-cm-diameter cylindrical applicator).

Dose target	TT	Gy (RBE)				
		Tumour Bed			Skull	Skin
Gy (RBE)	Min	Peak	Average	Minimum	Peak	Peak
10 as Peak	**2.0**	**10**	4.9	3.1	2.3	3.2
10 as Average	**4.0**	20.4	**10**	6.2	4.8	6.5
20 as Peak	**3.9**	**20**	9.8	6.1	4.7	6.4
20 as Average	**8.0**	40.8	**20**	12.5	9.5	13.0
50 as Peak	**9.8**	**50**	24.5	15.3	11.7	15.9
75 as Peak	**14.7**	**75**	36.7	22.9	17.5	23.9

It must be finally remarked that the results reported in previous figures and tables refer to:

- the brain cancers treatment. Nevertheless, these figures of merit could be generalised to the treatment of other solid tumours (e.g., breast[32]). Of course, for more accurate results, the topography of the tissues into (and around) the surgical cavity must be properly modelled;
- the nIORT® applicators with 4 and 6 cm in diameter. Different sizes could be employed by obtaining slightly different figures of merit. However, 4 cm is close to the minimum diameter allowed by the CNG architecture, that provides a sort of "switching on and off neutron brachytherapy tool" without using needles of radioisotopes. Diameters larger than 6 cm would be easily fabricated but, since the applicator is rigid, it would be challenging to adopt them with craniotomy, and more generally, for difficult achievable body parts such as pelvis and narrow cavities.

Discussion

The first advantage of IORT is the elimination of the interval between surgery and RT adjuvant treatments, the so-called time to initiation. Indeed, by its increase (as in the usual EBRT) there is a decreased efficacy in LTC of brain metastases for potential repopulation within the resection cavity walls. By the complete elimination of the time to initiation, IORT modalities may have the capacity to provide better outcomes than delayed EBRT approaches.

The fundamental aspect determining the IORT feasibility for the brain cancers treatment is the limited access to the tumour bed. The accessibility results easier with IOHDR needles and LEX-IORT devices with small spherical applicators, while it is more difficult for the IOERT cylindrical ones. This limitation should be partially avoided with the nIORT® device adopting the 4-cm-diameter applicator. At the same time, thanks to the neutron's diffusion, quite large target areas can be irradiated despite the small applicator diameter.

As described, for the correct choice of the most eligible IORT technique the physical features of the IR particles and their biological effects in the irradiated tissues must be considered. About the utilisation of neutrons as IR particles, it must be remarked

that the difficulties and limitations of the EBRT with fast neutrons[40,41,42] are mostly avoided by the IORT modality. And, despite the absence of clinical evidence, the nIORT® figures of merit can be "qualitatively" compared with those of the current techniques by referring to three parameters previously defined to characterise the IR beam.

a) The RBE value of 2.45 MeV energy neutrons is about 16: hence, nIORT® should be very effective in cancer cells killing. Indeed, using particles with high RBE (resulting from variations in LET along the IR path in tissues) the irradiation can be limited to the tumour volume with minimal normal tissue injury, and probably it will be also more efficacious against radioresistant tumours.[17] Even whether the underpinning radiobiological mechanisms are not still fully understood, there exists an increasing amount of data at the biochemical level concerning the IR effects due to accelerator-produced charged particles (as protons and heavy ions), but few data concerning the effects due to high-LET beams of fast neutrons. Nevertheless, the neutron radiobiology experiments clearly identified a higher cell kill per unit dose and an accompanying reduction in oxygen dependency.[43] Thanks to this high cells radiosensitivity and the limitation of the cancer cells repair (as discussed later), the neutron IR with high LET and RBE could also induce the killing of the motile cancer stem cells (CSCs) or metastatic CSCs (MCSCs) infiltrating the tumour bed (while X-rays and electrons with lower LET-RBE cannot lead to cells necrosis and, in some cases, induce these cells to develop radio-resistance).
b) The dose gradients in tissues depth are, at the same time, not steep as in the LEX-IORT treatment and less smooth than in the IOERT one. Thus, for a proper balancing between the LTC and the adverse effects of the radiation on normal tissues (and closest OARs, as the optic system), the peculiar nIORT® dose gradient profile could increase the IORT eligibility for the brain cancers treatment. As it results evident in Figs. 5 and 7, the overwhelming part of the dose is administered in the surface tissues and in the first cm depth, while in deeper tissues the levels are decisively lower (a factor @5 at 2÷2.5 cm depth).
c) The homogeneity of the dose level in the beam target area is well obtained with LEX-IORT and IOERT devices. Differently, the dose level on superficial tissues with the CNG-nIORT® device results higher in the centre and tends to decrease toward the tumour bed margins (see Figs. 4 and 6). This feature should avoid the adverse effects of the radiation on normal brain and neighbouring skin and skull tissues and, at the same time, should guarantee the LTC for the higher dose levels in the central area of the TME with residual disease. The almost-isotropic fast-neutron IR should allow to induce necrosis and apoptosis of the QCCs within the topography irregularities of the tumour bed and, maybe, also overcome their radio-resistance (responsible of multistage cancer progression and cancer metastasis too). Thanks to the neutron diffusion, the nIORT® results well suitable for large target areas and irregular surfaces, without the precise alignment required by IORT devices with focused beam IR particles. Furthermore, for extended tumour beds, it should not require the knowledge of the status of the surgical margins and lymph nodes before the treatment (as in standard IORT techniques).

Despite being a departure from the fractionated schemes of the EBRT, the nIORT® could satisfy all five R's criteria of radiotherapy, namely: radiosensitivity, reassortment, repopulation, reoxygenation and repair[44]. As mentioned before, the cells radio-sensitivity should be enhanced by the high LET and very high RBE of fast neutrons and the repopulation of residual cancer cells in the TME is not present because of the time to initiation zeroing in IORT treatments. Similarly, the reassortment - that in the EBRT is due to the rapid cells proliferation in which the heterogeneity in cell cycle kinetics redistributes (reassorts) cancer cells over the cycle between daily fractioned irradiations - does not play a role in a single-dose IORT irradiation delivered immediately after surgery. The reoxygenation effects needed to fix DNA damage (i.e., cancer cells are much more resistant to IR in hypoxic conditions) are enhanced by the IORT modality. In any case, the Oxygen Enhancement Ratio (OER) would be less affected by a hypoxic TME in case of neutrons: indeed, the nIORT® monoenergetic spectrum would set the OER to about the plateau of @1.45.[45]

For what concerns the cells repair, while photon radiation induces mainly isolated lesions including DNA SSBs, IR particles with high LET and RBE induces more highly localized DSBs and clustered DNA damage more difficult to repair, that should lead to necrosis and apoptosis of the cancer cells.[46] These lethal effects are counterbalanced by DSB-repair pathways that can act on DNA ends, such as the nonhomologous end joining (NHEJ), the homologous recombination (HR), the single strand annealing and theta-mediated end joining.[47,48,49] The size and complexity of DNA lesions inflicted by IR determine how the cells will be repaired. Mostly, the NHEJ pathway plays a predominant role in repairing

SSBs induced by low-LET radiation, while the slow DSB repair process that follows exposure to high-LET radiation is mediated by the "less efficient" HR process.[17] Furthermore:

- some trials indicated that the DNA of cancer cells repairs more slowly after RT treatments, which produce also more DNA breaks (single and double) than in normal cells, also because various proteins involved in cell death and DNA damage mechanisms decrease the radio-resistance of the fast-doubling cancer cells, while increase the radio-resistance of slow doubling normal cells[50,51];
- while the number of lethal DNA lesions for cancer cells (as DSBs and more complex lesions) is proportional to dose, the repair system of cancer cells becomes saturated at higher dose levels. Published evidences support the hypothesis that saturation of the repair system leads to increasing genomic instability that may contribute to inactivate tumour cells as the dose per fraction is increased beyond the dose range normally studied *in vitro*.[52] Furthermore, some preclinical studies suggest that DNA ends of DNA damage induced by high-LET IR are more prone to end processing compared to DNA ends of DNA damage induced by low-LET IR:[53] thus, despite the absence of clinical evidences, the fast neutrons of nIORT® could lead to some significant benefits.

It can be further observed that, when tested directly in cultured tumour cell populations, the radiation-induced death models for normal and cancer cells are very complex. A couple of complicating factors could be represented by the potential for:

- the radiation-induced bystander effect (RIBE) to increase the cell death beyond that which would be predicted. But, some preclinical studies evidence that the RIBE is not induced by neutrons[54] and thus a lower risk for RISMs should occur;
- the not-enough persistent capacity to impact tumour cell proliferation, epithelial-mesenchymal transformation (EMT) and transcriptional regulation. Analyses of tumour cavity wound fluids after IORT in breast cancer patients indicates encouraging results: although representing a drastically different TME, the potential of cytokine release and radiation immune modulation of the immune microenvironment of glioma in brain cancers has been hypothesized to play a role in potential IORT effects beyond radiation-induced DNA damage.[55,56]

The authors' idea is that the high RBE of fast neutrons could inhibit radically the cell proliferation and EMT in GBM cancers: if verified, this feature would be fundamental since EMT represent a crucial process endowing the cancer cells with invasive and metastatic properties, as well as radio-resistance. Additionally, thanks to the epithermal and thermal tails of the neutron flux spreading out around the tumour bed, the nIORT® could lead to the potential appearance of the radiation induced abscopal effect (RIAE) on distant non-irradiated cells due to the adaptive immune system.[57]

Nowadays investigations are focused on exploring combination therapies to mitigate undesirable side effects and enhance immune responses against tumours. The combination of immunotherapy with RT is an actively growing field of clinical investigation since the immune system can modulate either tumour suppression or progression, and RT has the potential to regulate immune responses to yield antitumorigenic effects leading to the tumour control.[58] The immunomodulatory impact of the RT to improve GBM outcome is still an open arena of research which requires further experimental and clinical trials.[59,60] However, there exists some clinical evidences that RT administered to oncological patients might augment the anti-tumour effects of administered immune-checkpoints inhibitors[61,62] (ICIs). This effect could also be performed – and hopefully enhanced - by the nIORT®: our hypothesis is that increasing the RBE of IR from 1 (for photons and electrons) up to 16 (for 2.45 MeV energy neutrons) should increase the immune modulatory effect by stimulating adaptive and innate immune reactions of the GBM oncological patients. Hence, in conjugation with the ICIs, the nIORT® could trigger a strengthen antitumor response and an improved patient survival.
Finally, it could be important to explore by preclinical studies whether the addition of concurrent and adjuvant TMZ[63,64] (an oral alkylating agent) to nIORT® could mitigate the resistance of the glioma stem cells (GSCs) in tumorigenesis and their function in the TME.

Conclusions

In this article, the 100kV-10mA DC CNG performances for potential nIORT® treatments of the GBM (and other brain cancers after craniotomy) have been accurately evaluated by the MCNP code, simulating the CNG equipped with two different cylindrical IORT applicators - 4 and 6 cm in diameter - and the surrounding surgical cavity (i.e., brain tumour bed, skull, and skin tissues). The high flux (~10^8 cm^{-2} s^{-1}), high LET and very high RBE (@16) of the 2.45 MeV neutrons beam allow to deliver equivalent dose rates ~5 Gy (RBE)/min in the centre of the tumour bed. Thus, the dose targets defined by the standard IORT clinical protocols - Boost, Radical and Ultra-Radical regimes - could be

administered in about 2, 4 and 10÷15 minutes, respectively. The tuneable size of the nIORT® adaptor permits to irradiate tumour beds with different extensions. The overwhelming part of the dose is released in tissues surface and in the first 1-2 cm depth, sparing normal tissues and the neighbouring OARs from harmful radiations. The almost spatial-isotropic diffusion of neutrons allows to irradiate tumour beds with irregular surfaces (without stringent beam focusing requirements, as in the IOERT and in the proton therapy) and the surrounding potential QCCs in the surgical cavity, that should lead them to necrosis and apoptosis by increasing the LTC and reducing the chances of local recurrence.

Thus, for patients affected by GBM and other brain cancer malignancies, the irradiation by a nIORT® clinical device - during the maximum safety surgical resection - could become a standard-of-care adjuvant treatment, eventually in combination with other adjuvants as chemotherapy and/or immunotherapy. Of course, as here discussed, these hypotheses will have to be validated by *in vitro* and, especially, *in vivo* pre-clinical tests.

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Ethical Statement

The authors are accountable for all aspects of the work and contributed equally to this research.

Author contributions

Martellini Maurizio, Sarotto Massimo, Leung Ka-Ngo, Gherardi Giuseppe, Rizzo Antonietta, Ottaviano Giuseppe and Falzone Lidia contributed equally to this work.

Conflicts of Interest

Authors Martellini Maurizio, Sarotto Massimo, Leung Ka-Ngo, Gherardi Giuseppe, Rizzo Antonietta, Ottaviano Giuseppe and Falzone Lidia declare that they have no conflicts of interest.

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