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Original paper

NOD-SCID mice irradiation with medical accelerators: Dosimetric and radiobiological results

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ABSTRACT

Purpose: Preclinical studies normally requires dedicated instruments due to the small anatomical scales involved, but the possibility of using clinical devices for this purpose may be of economical, scientific and translational interest.

In the present work the accurate description of treatment planning, dosimetric results, radiotoxicity and tumor response of the irradiation of NOD-SCID mice were presented. Two medical linear accelerators, TrueBeam STx and Tomotherapy Hi-ART, were compared. NOD-SCID mice irradiation with Tomotherapy is a novelty, as well as the comparison of different irradiation techniques, devices and dose fractionations.

Methods: Human derived glioblastoma multiforme neurospheres were injected in immunocompromised NOD-SCID mice to establish xenograft models. Mice were anaesthetized and placed in a plexiglas cage pieboth to perform CT scan for treatment planning purposes and for the irradiation. Three fractionation schedules were evaluated: 4 Gy/1 fraction, 4 Gy/2 fractions and 6 Gy/3 fractions. Tomotherapy planning parameters, the presence of a bolus layer and the irradiation time were reported.

After irradiation, mice were examined daily and sacrificed when they showed signs of suffering or when tumor volume reached the established endpoint. Outcomes regarding both radiotoxicity and tumor response were evaluated comparing irradiated mice as respect to their controls.

Results: Survival analysis showed that Tomotherapy irradiation with 6 Gy/3 fractions with a bolus layer prolong mice survival (log-rank test, $p < 0.02$). Tumor volume and mice survival were significantly different in irradiated xenografts as compared to their controls (*t*-test, $p < 0.03$; log-rank, $p < 0.05$).

Conclusion: The radiobiological potential of Tomotherapy in inducing tumor growth stabilization is demonstrated.

1. Introduction

Preclinical studies are critical steps in the medical research process, and small animal irradiation is one of the delicate fields in which the different anatomical scales could significantly affect the results and the technique may do the difference. The small animal size, in the order of magnitude of 3 cm of maximum diameter for about 20 g of weight, generates problems related to submillimetrical organ separation and, at the beam energy of 6 MV, to dose deposition into tissues. Moreover, in order to avoid deep anesthesia and related side effects, total irradiation time should be as short as possible.

The small volumes involved in the irradiation may necessitate of dedicated instruments such as kV X-ray generators and Ir-192 sources [1]. In the past years, many radiotherapy departments had orthovoltage X-ray treatment machines, suitable for superficial target irradiation; more recently these devices have been replaced by modern, *high energy*, linear accelerators, therefore data presented in this paper are related to the most common irradiation options nowadays. Furthermore, the possibility of dose conformation of Ir-192 is lower as compared to external beam irradiation. For those centers in which both preclinical research and clinical diagnostics and treatments are conducted, the dosimetric feasibility of the use of clinical devices for preclinical purposes may be of economical, scientific and translational interest [1].

Translational power of preclinical experiments is also limited by the possibility of simulating human fractionation on mice. In fact, the lower total dose tolerance of NOD-SCID mice limits the

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dose per fraction as well as the total number of fractions of preclinical experiments. Besides mice radiosensitivity, total duration of preclinical experiments may also be a limiting factor.

Our work aims to show dosimetric and radiobiological results of the irradiation of NOD-SCID mice by using two medical linear accelerators, TrueBeam STx and Tomotherapy. In particular, the accurate description of treatment planning, dosimetric results, radiotoxicity and tumor response in NOD-SCID mice irradiated with Tomotherapy is a novelty, as well as the comparison of different irradiation techniques, devices and fractionations on NOD-SCID mice. Presented tumor response results are obtained on mice inoculated with highly radioresistent neurospheres [2], and the possibility of irradiating mice with the same dose per fraction in a fractionated irradiation scheme is investigated.

2. Materials and methods

2.1. Xenograft models

All animal procedures were approved by the internal Ethical Committee for Animal Experimentation (FPRC-CESA) and by the Italian Ministry of Health.

Glioblastoma neurospheres were derived and cultured as described by De Bacco F et al. [2,3]. In particular, data presented in this work are obtained studying xenografts established by injecting three different human-derived neurospheres: BT308 [2,3] and BT371 [2] to generate subcutaneous xenografts and BT463 [2] to generate orthotopic xenografts.

To establish xenograft models, single-cell suspensions of glioblastoma neurospheres were injected subcutaneously or intracranially into 6–8 weeks old female NOD.CB17-Prkdc^{scid}/NcrCr mice (Charles River Laboratories).

To obtain subcutaneous xenografts, 2×10^5 viable cells in 100 µl v/v PBS/Matrigel (BD Biosciences) were injected into the right flank of the mice. Once the tumors were established (average volume equal to 100–300 mm^3, depending on experimental model) mice were randomized into two groups: untreated controls and irradiated (IR, 6 Gy total dose, fractionated in 2 Gy doses given for 3 consecutive days). Tumor diameters were measured by caliper and tumor volume was calculated using the formula: $4/3\pi \times (d/2)^2 \times (D/2)$, where d and D are the minor and the major tumor axis, respectively.

For orthotopic xenografts, 2.5×10^5 viable luciferase-gfp expressing cells in 2 µl of PBS were delivered into the corpus striatum of the right hemisphere by stereotactic injection (coordinates used were as follows: antero-posterior = +0.8 mm; medio-lateral = +2.0 mm; dorso-ventral = −3.0 mm). Intracranial tumor growth was monitored by bioluminescence (BLI) imaging (IVIS® SpectrumCT, Caliper Life Sciences). For bioluminescence imaging, luciferin (D-Luciferin potassium salt, Caliper Life Sciences) was dissolved in PBS (150 mg/kg) and administered to mice by subcutaneous injection. Anesthesia was delivered in an induction chamber with 2.5% isofluorane in 100% oxygen at a flow rate of 1 L/min and maintained in the IVIS with a 1.5% mixture at 0.5 L/min. Based on BLI signal, mice were randomized in two groups and treated as described above.

2.2. Xenografts irradiation setup

Both subcutaneous and pan-encephalically irradiated mice were anesthetized by i.p. anesthesia (zoletil 40 mg/kg + xylazine 7.5 mg/kg) and placed in a plexiglass pie cage (2Biological Instruments).

For treatment planning purposes, CT scans were acquired and reconstructed with a slice thickness equal to 3 mm by a Toshiba Aquilion LB scanner (Toshiba Medical Systems Corp.). Target volumes (Clinical Target Volume, CTV; Planning Target Volume, PTV) and organs at risk (OARs) were delineated on CT scans.

For subcutaneous models, in order to detect the location of the tumor, lead markers were applied on mice's skin. For each mouse, CTV was delineated in the low-abdomen, in a region corresponding to the area of subcutaneous tumor. Lungs (L) and gastrointestinal tract (GI) were considered OARs. To set the PTV, a margin of 1 or 2 mm was defined depending on the proximity of the target to OARs (Fig. 1, left).

Concerning pan-encephalic irradiation, the CTV corresponds to total brain volume and a ring-shaped target structure, comprising mice's heads, was delineated on the purpose of the treatment planning. Lungs were considered as OARs (Fig. 1, right). Body (B) volume was also considered for dosimetric analysis.

For irradiation, two different machines were used: a TrueBeam STx (TB, Varian Medical Systems, Inc., Palo Alto, USA), equipped with the HD 120 *microMultiLeaf* (MLC, 0.25 cm leaves) and a Hi-Art Tomotherapy system (Accuray Inc., Sunnyvale, CA), equipped with 0.65 cm leaves. The energy of both radiation beams is 6 MV, with an average build-up thickness equal to about 1.5 cm. Treatment planning was performed on dedicated Treatment Planning Systems (TPS): Eclipse (Varian Medical Systems, Inc., Palo Alto, USA) for TB planning, with the *Anisotropical Analitical Algoritm* calculation algorithm, and Tomotherapy TPS, with the *Collapsed Cone Convolution* calculation algorithm. For Tomotherapy, TomoDirect Intensity Modulated Radiation Therapy (TD-IMRT) technique with inverse-planning approach, was used, with gantry fixed at 0° and 180°. Field Width (FW), Pitch and Modulation Factor (MF) have been chosen as further planning parameters for each single irradiation. The actual MF is evaluated by the TPS during the final dose calculation. For TB a 3DCRT technique, related to a forward planning approach, was used: the gantry was also fixed at 0° and 180° with a static MLC conformation.

In order to obtain a better coverage to deposit radiation also in the superficial layer and a better overall control of the dose, in some experiments, a 0.6 cm bolus layer (Elasto-gel) has been placed upon mice's body for subcutaneous tumors and upon mice's head for intracranial models. To determine the importance of this expedient, where indicated, planning and dosimetric results obtained from mice irradiated with bolus (WB) have been compared to those obtained from groups of mice irradiated without bolus (WOB).

Before the delivery of each Tomotherapy RT fraction, a Megavoltage Computed Tomography (MVCT) image has been acquired in order to correct setup of the mice. Setup of mice irradiated with TB was settled with light field. After irradiation mice were

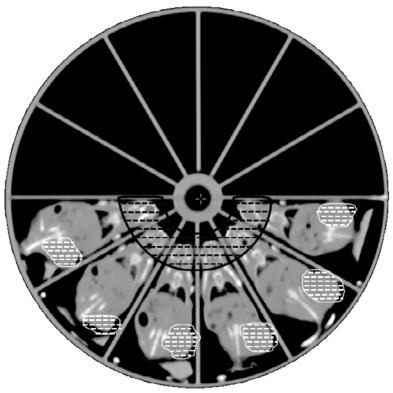

Fig. 1. Coronal CT slice of the cage pie containing 6 subcutaneous models: target volume is highlighted with white dotted lines; the target structure normally delineated for pan-encephalic irradiation is also displayed with black dotted lines.