

Development of a low-energy particle irradiation facility for the study of the biological effectiveness of the ion track end

This article has been downloaded from IOPscience. Please scroll down to see the full text article.

2012 J. Phys.: Conf. Ser. 373 012019

(<http://iopscience.iop.org/1742-6596/373/1/012019>)

View [the table of contents for this issue](#), or go to the [journal homepage](#) for more

Download details:

IP Address: 192.84.134.230

The article was downloaded on 22/11/2012 at 16:45

Please note that [terms and conditions apply](#).

Development of a low-energy particle irradiation facility for the study of the biological effectiveness of the ion track end

L Manti¹, L Campajola¹, FM Perozziello¹, JN Kavanagh², G Schettino²

¹Dipartimento di Scienze Fisiche, Università di Napoli “Federico II”, Complesso Universitario Monte S. Angelo, 80126, Napoli, Italy

²CCRCB, Queen’s University Belfast, 97 Lisburn Road, BT9 7BL, Belfast, Northern Ireland, UK

E-mail: g.schettino@qub.ac.uk

Abstract. Uncertainties surround the radiobiological consequences of exposure to charged particles, despite the increasing use of accelerated ion beams for cancer treatment (hadrontherapy). In particular, little is known about the long-term effects on normal tissue at the beam entrance or in the distal part of the Spread-Out Bragg Peak (SOBP). Moreover, although the relative biological effectiveness (RBE) of particle radiation has been traditionally related to the radiation linear energy transfer (LET), it has become increasingly evident that radiation-induced cell death, as well as long term radiation effects, is not adequately described by this parameter. Hence, exploring the effectiveness of various ion beams at or around the Bragg peak of monoenergetic ion beams can prove useful to gain insights into the role played by parameters other than the particle LET in determining the outcome of particle radiation exposures. In this context, the upgrade of the Tandem irradiation facility at Naples University here described, has allowed us to perform a series of preliminary radiobiological measurements using proton and carbon ion beams. The facility is currently used to irradiate normal and cancer cell lines with ion beams such as oxygen and fluorine.

1. Introduction

Ion beams have been used for radiotherapy applications for some years [1], [2], [3] with protons used in many facilities worldwide while heavier ions (i.e. carbon) have been exploited in Japan and Germany with impressive outcomes [4]. Despite these encouraging results, there are still considerable uncertainties regarding the biological effects caused by exposure to charged particles. A deeper understanding of the effects caused by heavy ions on biological samples is therefore essential to develop a rigorous theory of ion radiation action at the cellular and molecular level and improving tumour hadrontherapy.

It is now commonly accepted that the biological effectiveness of charged particles is critically determined by the pattern of ionizations produced by the ion traversal (i.e. track structure) [5]. While the linear energy transfer (LET) is a good indicator of the rate of energy deposition, at extremely high and inhomogeneous LET regions it does not predict biological effects with a simple relationship [6-8]. Radiotherapy studies investigating the biological response of different cell lines to different types of charged particles have focused mainly on the cell killing effect on tumour cells in the so-called Spread Out Bragg Peak (SOBP) [9-11]. However, effects caused in tissues at the beam entrance channel, in the distal part of the SOBP and in the surrounding areas also need to be addressed. Moreover, the

increasing interest in utilizing a range of ions, including light ions other than protons, such as boron or lithium, and ions heavier than carbon in therapy [12], demands further development of suitable models (such as the Local Effect Model) to measure and predict the effectiveness of these ions in biological systems.

In this manuscript, we report the upgrading of the radiobiological irradiation facility at the University of Naples to include ions other than protons. Details of the experimental set-up including ion source, dose and energy characterization are reported together with first biological measurements of cell survival, bystander effects and radiation-induced premature cellular senescence in normal human fibroblasts (AG01522) exposed to a proton ion beam. Survival curves following carbon ion irradiation of a panel of glioma human cell lines of varying radiosensitivity are also reported.

2. Materials and Methods

2.1. Irradiation set-up

The radiobiological irradiation facility at the Department of Physical Sciences of the University of Naples “Federico II” is centered on a 3-MV HVEC TTT-3 Tandem accelerator. TiH₂ and graphite cathodes and a negative ion sputtering source are used to produce hydrogen and carbon beams, respectively, which can then be accelerated up to 6 MeV for protons and 21 MeV for carbon. Lithium (Li) and Fluorine (F) ion beams have also been produced using LiF cathodes and accelerated up to 12 MeV and 21 MeV, respectively. Finally, oxygen beams were produced by spraying O₂ gas onto a tantalum cathode; they can be accelerated up to 21 MeV. Due to energy loss in the scattering device and during extraction from vacuum, the maximum ion energy incident onto the cells is slightly lower as indicated in table 1. The beam intensity is monitored by several Faraday cups placed along the beam line and a fast computer controlled beam shutter is positioned after the last cup in order to precisely control the dose delivered to the biological samples. Downstream, a 1 mm collimator is used to focus the beam on the scattering chamber where an Au foil (0.22 μm) scatters the beam achieving the required homogeneous irradiation at the end of the beam line (approximately 2 m away from the scattering chamber, see figure 1). Proton irradiation was performed using the described set-up, in which the beam passed through a 6 μm Mylar window, a 2 mm air gap and the Mylar base (2.5 μm) on which cells were grown. In order to minimize energy loss and thus make it possible to use also heavier ion beams for radio-biologically relevant experiments, the Mylar substrate on which the biological samples are seeded was reduced to 1.5 μm and also used as vacuum window at the end of the beam line. In this way, the air gap and relative uncertainty on energy loss is abolished. This requires a differential vacuum section at the end of the beam line, which can be pumped independently from the main beam line allowing for positioning and removal of the biological samples (figure 1). To prevent mechanical stress on Mylar foil, this is laid upon a tungsten grid of 50 μm wires, which translated in 6% opacity on the irradiated surface.

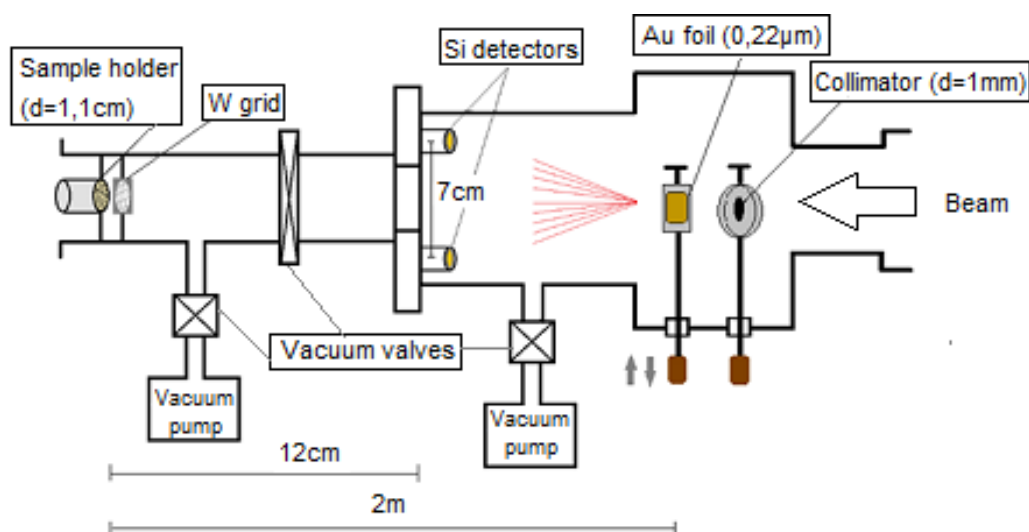


Figure 1. Schematic diagram (not to scale) of the beam line end with differential vacuum sections and the biological sample holder.

2.2. Dosimetry and dose monitoring

Energy spectra and dosimetric measurements are performed 2 m away from the scattering chamber by using Si-based detectors as well as CR39 polycarbonate solid state track detectors. Three Si-based detectors (300 μm thick and 25 mm² sensitive area, 14 keV resolution) are mounted at the end of the beam line. The first one is centred with the beam axis and is therefore positioned in the same place where the biological samples will be during the irradiation. This detector is used to characterise the irradiation beam (particle type, energy, fluency) and it is removed during the sample irradiation. The two other Si-based detectors are placed symmetrically at 3.5 cm from the beam axis and are permanently mounted at the end of the beam line for continuously monitoring the dose rate during irradiation. All three Si-based detectors are cross-calibrated in order to estimate beam uniformity and dose delivered to the biological samples. CR39 solid-state track detectors (SSTDs) positioned at the irradiation spot using the biological sample holders (see below) are also used to verify and cross-calibrate the Si-based detector response. Pre- and post-irradiation CR39 counts performed on at least 10 random fields at light microscope (32X, corresponding to a field area of 1.25 10⁻⁴ cm²) were used to evaluate the Si-based detector calibration factor and eventually correct retrospectively nominal dose values.

The dose absorbed by the biological sample is calculated using the following formula:

$$\text{Dose (Gy)} = \text{Fluence (particles/cm}^2\text{)} \times \text{LET (keV/}\mu\text{m)} \times 1.6 \cdot 10^{-9}.$$

Particle fluence and beam uniformity were measured and monitored by means of the CR-39 SSTDs. From it, administered doses were retrospectively calculated using the calibration factor between Si detector counts and measured mean particle numbers per cm² from the CR39 plastics. Etching was carried out by KOH 10N at 80 °C for 30 min. Calculated uniformity across the Mylar base (irradiation field) was 7%. Typical dose rates ranged between 0.5 and 2 Gy min⁻¹.

2.3. Sample preparation

Custom-made pyrex glass cylindrical wells were glued onto a well stretched Mylar foil (1.5 μm) by means of araldite® and cured in oven at 140 °C for 4 h. Two days prior to irradiation cells from a single-cell suspension were seeded at about 4 10⁴ cells/well in 2 ml growth medium. A cell monolayer was thus obtained on the day of exposure. At the moment of irradiation the medium was aspirated and a sterile cap placed on the cylinder which was then placed horizontally in the sample holder.

For the clonogenic measurements of directly irradiated samples, cells were washed with PBS and trypsinized from the Mylar based wells straight after the irradiation using 0.5 ml of 0.025% trypsin-EDTA incubated at 37°C for 5-10 minutes. The sample density of the resulting single cell solution was estimated by hemocytometer counting and the desired number of cells plated in T25 plastic flasks. After 14 days incubation at 37°C, 5%CO₂, colonies were fixed and stained using crystal violet solution (0.05% w/v in methanol) for manual scoring. For the bystander measurements, 2 ml medium was removed from the irradiated wells and added to cells in unexposed wells through a 0.2 µm filter straight after the irradiation. The bystander unexposed wells were then incubated at 37°C for 1 hr before following the trypsination and reseeding protocol described above.

For radiation-induced senescence experiments, cultures were propagated and tested at time intervals by means of commercially available kits (β-galactosidase assay): briefly, cells seeded in 35-mm petri dishes, fixed in paraformaldehyde and incubated in β-galactosidase (β-gal) staining solution overnight at 37 °C in the absence of CO₂. Scoring of senescent cells (positive for β-gal) was performed by means of light microscopy sampling random fields: senescent cells appeared brightly green. At least 500 cells per sample were scored.

The cell lines used for the preliminary experiments reported in this manuscript were 3 tumor cell lines (U87MG, T98G and LN229) all derived from human glioblastomas, differing slightly in their genetic make-up (i.e. tp53 status) and a normal human fibroblast cell line (AG01522). These specific cell lines were chosen as glioblastomas are one of the human tumors most suitable for hadrontherapy treatment due to their radio-resistance and difficulty in removal by surgery. On the other hand, fibroblasts are commonly found as connective and supportive tissue in many parts of the body and are inevitably exposed during external beam radiotherapy.

3. Results

3.1. Ion energy

Protons of nominal energy $E = 4$ MeV were accelerated by a terminal voltage V_T of 2 MV. ¹²C ions were produced with $q=5+$ ($V_T = 2.4$ MV). From measured spectra, H and ¹²C beams emerged from Au foil with energies of 3.5 MeV and 13.7 MeV, respectively. An example of a measured spectrum after the Au foil is shown in figure 2. Further energy loss due to the Mylar sheet on which cells are seeded and, for protons, an air gap, resulted in incident energy on cells of 3.3 MeV for protons and 12.4 MeV for carbon ions.

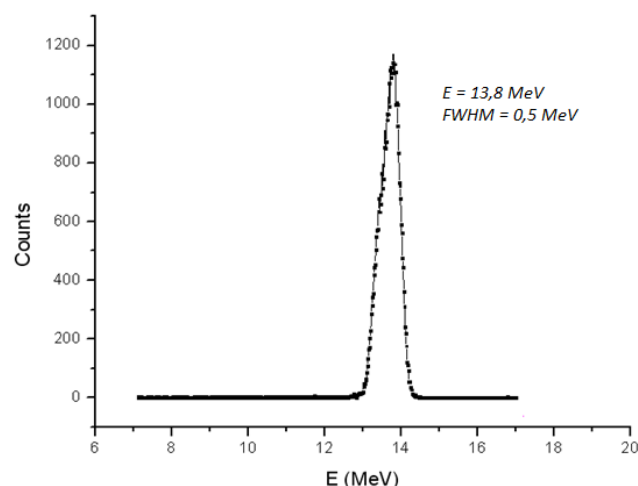


Figure 2. Measured spectrum for ¹²C ion beam reaching the biological samples.

3.2. LET calculations

Incident energy, LET values and residual ranges were estimated using SRIM 2011 (www.srim.org) and results are reported below.

Table 1. Range of incident energies and LET values for cellular irradiation

Ion	Incident Energy [MeV]	Incident LET [keV/ μm]	Residual Range in Water [μm]
p	3.3	11	174
^{12}C	12.4	730	16
^{16}O	15.5	1,100	15
^{19}F	16.7	1,350	14

As indicated in figure 2, the energy spread measured as FWHM is <5% of the mean ion energy which translates into an uncertainty in the LET calculations of ~3%. It must be noted that the LET values reported are those incident (i.e., at the surface of the biological sample).

3.3. Proton Irradiation

3.3.1. Cell survival

Survival measurements from normal human fibroblasts cells (AG01522) directly exposed to monoenergetic 3.3 MeV proton beams have been compared to data from clonogenic experiments performed with 225 kVp X-rays and 62 MeV proton from the INFN-LNS facility (figure 3). For the latter, the beam was modulated to obtain a SOBP as used for clinical applications [13] and cells exposed to the centre of the SOBP. For the X-rays and the 62 MeV proton experiments, cells were irradiated in plastic flasks but similar trypsinization and re-seeding procedure were followed.

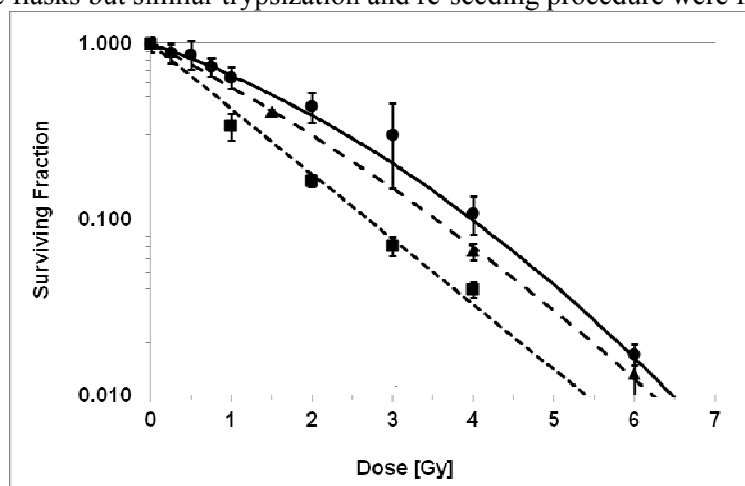


Figure 3. AG01522 survival following exposure to 3.3 MeV protons (■) and centre of SOBP for a 62 MeV proton beam (▲). 225 kVp X-ray survival curve is also reported (●).

RBE values at 10% survival ($\text{RBE}_{0.1}$) were calculated from the α and β parameters obtained fitting the experimental data with the linear quadratic model.

Table 2. RBE values for AG01522 cells irradiated with proton beams.

Proton Energy [MeV]	LET [keV/ μm]	$\text{RBE}_{0.1}$
3.3	11	1.57 ± 0.07
62 - Centre SOBP	4	1.14 ± 0.02

3.3.2. Bystander effect

AG01522 cells were also used for investigation of the bystander response following 3.3 MeV proton exposures. Survival data for the bystander experiments are reported in figure 3 as a function of the dose received by the directly exposed cells from which the media was transferred.

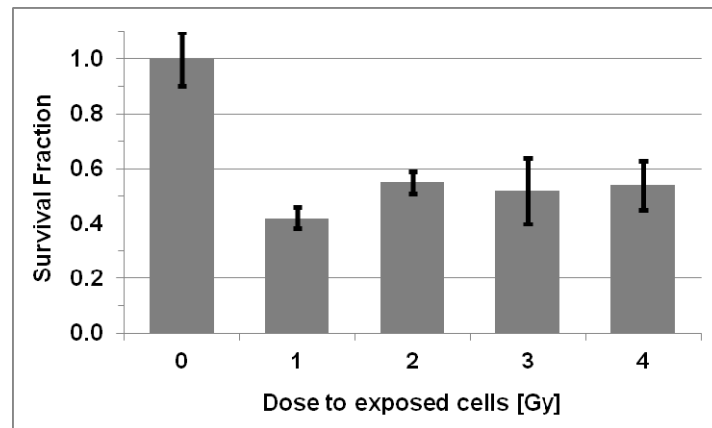


Figure 3. Survival following media transfer from AG01522 exposed to 3.3 MeV protons.

3.3.3. Senescence

The onset of cellular senescence was assayed both as an early and a late-occurring response to 3.3 MeV proton irradiation (figure 4).

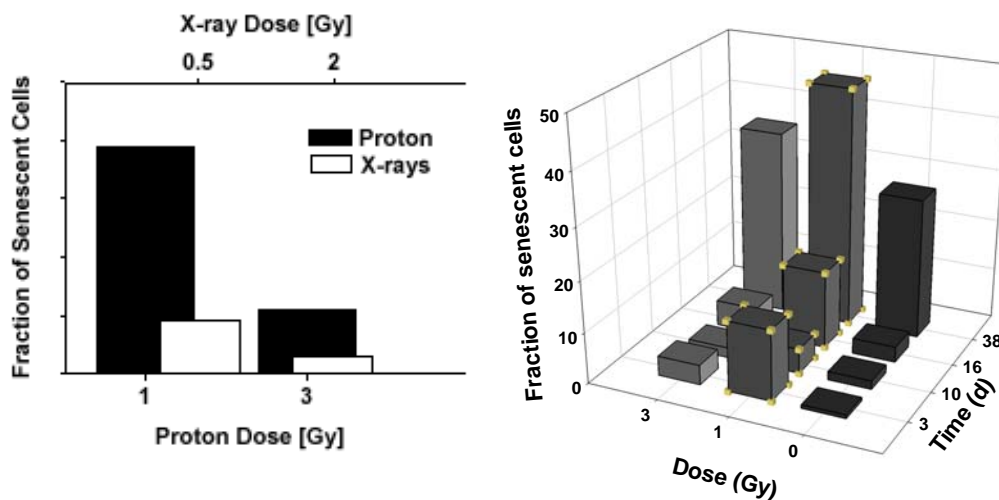


Figure 4. Early (72 hrs, left plot) and late (up to 38 days, right plot) cellular senescence post 3.3 MeV proton exposure. X-ray data are also reported for comparison.

3.4. Carbon

3.4.1. Cell survival

The biological effectiveness of proximal-to-Bragg peak (~16 μm before end of range) Carbon ion beam was investigated by measuring the survival fraction of 3 glioma cell lines (figure 5). No statistically significant differences were observed between the 3 cell lines with an estimated $\text{RBE}_{0.1}$ of ~1.7 compared to conventional X-ray beams (data not included).

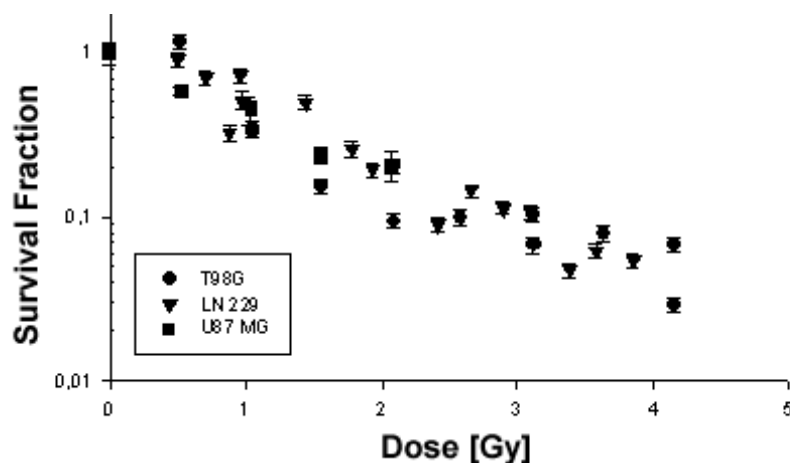


Figure 5. Cell survival of glioblastoma cell lines exposed to 12.4 MeV ^{12}C beam

4. Discussion

In this manuscript we report the successful upgrade of the radiobiology beam line facility at the University of Naples (Italy) with inclusion of a wide range of ion species. Carbon, Oxygen and Fluorine have been added to protons as beams that can be extracted into biological samples through a differential vacuum chamber and thin Mylar substrates. Field uniformity and dose rate are suitable for a wide range of radiation biology investigations. The maximum energy available is such to allow the heavier ions to just penetrate a single cell layer. This makes the facility ideal to investigate the biological effectiveness of the final part of the ion tracks reporting cellular effects as a function of the incident LET (as the LET varies drastically within the cell at these energies) and the atomic number (Z) of the ion beam. The effectiveness of the final part of ion tracks is critical for the optimization of RBE values to be used in clinical settings as large effects are expected and cancer cells as well as “healthy” cells may experience such traversals.

Initial biological experiments have been performed with proton and carbon beams using normal and cancer human cell lines. Survival fractions of normal human fibroblast cells (AG01522) irradiated with 3.3 MeV protons have been compared to data obtained following exposures to conventional X-ray and modulated energetic proton beams (centre of SOBP of 62 MeV protons from the INFN-LNS). Data indicate a significantly higher RBE (~ 1.6) for the 3.3 MeV protons compared to the centre of the SOBP (~ 1.1) highlighting how fixed RBE values for clinical modulated beams is an oversimplification, and considerable enhancement is to be expected in the distal part of the SOBP [14]. Interestingly, our bystander investigation shows a considerable reduction in the clonogenic potential of cells receiving media from irradiated samples. The magnitude of the effect ($\sim 50\%$ survival reduction) could be due to the high density of cells irradiated ($\sim 10^5$ cells in 2 ml) suggesting that signal concentration could modulate the bystander response. Finally, our proton data show cellular senescence significantly elevated as early as 72 hr post irradiation, contrary to what was observed with X-rays. Moreover, the level of senescent cells is constantly found elevated among proton-irradiated cultures compared to controls for up to over a month post exposure. Interestingly, at lower dose there is a higher occurrence of senescent cells which is consistent with cellular senescence as a sub-lethal stress response and in accordance with our previous results with carbon ion beams [15]. Consequences of non-lethal stress responses are of considerable interest as long term late effects, including secondary cancers, will ultimately determine the outcome of radiotherapy treatments.

Experiments with carbon beams aimed at quantifying the lethal effects of the ^{12}C track end in a panel of human glioma cell lines. No significant differences between the 3 cell lines used (U87MG, T98G and LN229) have been observed. When compared to X-rays, an RBE of ~ 1.7 can be estimated. This is considerably lower than what is expected in the centre of a clinical modulated carbon beam but in line with the RBE decrease at very high LETs (i.e., 700 keV/ μm) [16]. In fact, according to the

classical overkill effect, at such very high LET values, RBEs ought to be close to 1, hence our data may imply inadequacy of LET to describe complex effects near the Bragg peak possibly determined by the track structures. Future studies will be aimed at investigating the biological effectiveness of different types of ions with similar LET characteristics. This will provide insights into the role of delta electrons in determining the biological response to ion exposures.

References

- [1] Yuh, G E, Loredò L N, Yonemoto LT, Bush D A, Shahnazi K, Preston W, Slater J M and Slater J D, Reducing toxicity from craniospinal irradiation: using proton beams to treat medulloblastoma in young children. *Cancer J.* 2004 10 386-90
- [2] Kamada T, Tsujii H, Tsuji H, Yanagi T, Mizoe J E, Miyamoto T, Kato H, Yamada S, Morita S, Yoshikawa K, Kandatsu S and A. Tateishi, Efficacy and safety of carbon ion radiotherapy in bone and soft tissue sarcomas. *J. Clin. Oncol.* 2002 20 4466-71
- [3] Imai R, Kamada T, Tsuji H, Yanagi T, Baba M, Miyamoto T, Kato S, Kandatsu S, Mizoe J E, Tsujii H and Tatezaki S, Carbon ion radiotherapy for unresectable sacral chordomas. *Clin. Cancer Res.* 2004 10 5741-6
- [4] Jereczek-Fossa B.A., Krengli M and Orecchia R Particle beam radiotherapy for head and neck tumors: radiobiological basis and clinical experience. *Head Neck* 2006 28 750-60
- [5] Jenner T J, Delara C M, O'Neill P and Stevens D L, Induction and rejoining of DNA double-strand breaks in V79-4 mammalian cells following gamma and alpha irradiation. *Int. J. Radiat. Biol.* 1993 64 265-273
- [6] Thacker J, Stretch A and Stephens M A, Mutation and inactivation of cultured mammalian cells exposed to beams of accelerated heavy ions. II. Chinese hamster V79 cells. *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* 1979 36 137-48
- [7] Cucinotta FA, Wilson JW, Shavers M R and Katz R, Effects of track structure and cell inactivation on the calculation of heavy ion mutation rates in mammalian cells. *Int. J. Radiat. Biol.* 1996 69 593-600
- [8] Durante M, K. George K, Gialanella G, Grossi G, La Tessa C, Manti L, Miller J, Pugliese M, Scampoli P and Cucinotta F A, Cytogenetic effects of high-energy iron ions: dependence on shielding thickness and material. *Radiat. Res.* 2005 164 571-6
- [9] Kanai T, Endo M, Minohara S, Miyahara N, Koyama-Ito H, Tomura H, Matsufuji N, Futami Y, Fukumura A, Hiraoka T, Furusawa Y, Ando K, Suzuki M, Soga F and Kawachi K, Biophysical characteristics of HIMAC clinical irradiation system for heavy-ion radiation therapy. *Int. J. Radiat. Oncol. Biol. Phys.* 1999 44 201-10
- [10] Furusawa Y, Fukutsu K, Aoki M, Itsukaichi H, Eguchi-Kasai K, Ohara H, Yatagai F, Kanai T and Ando K, Inactivation of aerobic and hypoxic cells from three different cell lines by accelerated ^3He -, ^{12}C - and ^{20}Ne -ion beams. *Radiat. Res.* 2000 154 485-96
- [11] Uzawa A, Ando K, Koike S, Furusawa Y, Matsumoto Y, Takai N, Hirayama R, Watanabe M, Scholz M, Elsässer T and Peschke P, Comparison of biological effectiveness of carbon-ion beams in Japan and Germany. *International Journal of Radiation Oncology, Biology & Physics* 2009 73 1545-51
- [12] Brahme A, Recent advances in light ion radiation therapy. *Int. J. Radiat. Oncol. Biol. Phys.* 2004 58 603-16
- [13] Spatola C, Privitera G, Raffaele L, Salamone V, Cuttone G, Cirrone P, Sabini M G and Lo Nigro S, Clinical application of proton beams in the treatment of uveal melanoma: the first therapies carried out in Italy and preliminary results (CATANA Project). *Tumori* 2003 89 502-9
- [14] Paganetti H, Niemierko A, Ancukiewicz M, Gerweck LE, Goitein M, Loeffler J S and Suit H D, Relative biological effectiveness (RBE) values for proton beam therapy. *Int. J. Radiat. Oncol. Biol. Phys.* 2002 **53** 407-21
- [15] Grossi G F, Bettega D, Calzolari P, Durante M, Elsässer T, Gialanella G, Hessel P, Manti L, Pugliese M, Ritter S, Santini M T, Scampoli P and Weyrather W K, Late cellular effects of ^{12}C ions *Il Nuovo Cimento C* 2008 31 39-47
- [16] Sorensen B S, Overgaard J and Bassler N, In vitro RBE-LET dependence for multiple particle types. *Acta Oncol.* 2011 **50** 757-62