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RBE predictions by the BIANCA model for *in vitro* and *in vivo* irradiations in hadron therapy scenarios

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Summary. — BIANCA is a two-parameter biophysical model that simulates chromosome aberrations and cell death induced by ionizing radiation, including ions of interest for hadron therapy. In this framework, the model parameters were tuned to produce a radiobiological database describing the survival of V79 cells, chosen as a reference, after irradiation with different monochromatic ion beams. Afterwards, an approach was developed to produce analogous databases for other cell lines, starting from their photon response; this approach allows performing full predictions for, in principle, any cell line. These databases can be read by a radiation transport code or a treatment planning system (TPS). Recently, BIANCA was interfaced to the FLUKA Monte Carlo code, and was applied to predict cell survival and RBE in typical hadron therapy scenarios. Very good agreement was found with *in vitro* data on the survival of CHO cells exposed along Spread Out Bragg Peaks of protons, C- and He-ions. Furthermore, good agreement was obtained with *in vivo* RBE data on late effects in the rat spinal cord following irradiation with C-ions or protons. Finally, BIANCA was applied to two C-ion patient cases, showing predictions in line with other two models currently applied in clinics (LEM I and MKM). This work thus suggests that BIANCA can be used to predict RBE for hadron therapy.

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1. – Introduction

Cancer hadron therapy is a particular form of radiotherapy, which makes use of particles like protons or carbon ions and presents two main advantages. The first one is due to the physical properties of charged particles which, depositing most of their energy at the end of their paths, allow obtaining a highly conformal dose distribution in the tumor. The second advantage relies on the higher biological effectiveness of ions heavier than protons with respect to photons, leading to a higher efficacy in treating radioresistant tumors, such as head-and-neck tumors [1].

The enhanced biological damage induced by these ions can be quantified by means of the so-called Relative Biological Effectiveness (RBE). This quantity depends on many factors, including radiation quality (that is particle type and energy), considered endpoint, dose level and cell (or tissue) radiosensitivity. In general, the RBE varies along the particle beam and along the SOBPs (Spread Out Bragg Peaks) of hadron therapy treatments, and thus needs to be accurately predicted in each point of the treatment field, in order to correctly assess the biological impact of the treatment.

RBE prediction for heavy ions can be performed by biophysical models. Currently, only three models are applied in clinics: the Local Effect Model (LEM) [2] in Europe and Shanghai (China), the Microdosimetric Kinetic Model (MKM) [3] and the "mixed-beam" model [4] in Japan. In order to be applied in clinics, a biophysical model needs to be validated against both *in vitro* and *in vivo* data. Concerning the latter, LEM was for example benchmarked against data on the tolerance of the spinal cord of rats irradiated with C-ion beams [5, 6]. This endpoint is considered as a reference for late reactions in the CNS (Central Nervous System), which represent the main dose-limiting factor for head-and-neck tumor treatments.

In Italy, at the University of Pavia and at INFN (Istituto Nazionale di Fisica Nucleare), another biophysical model, called BIANCA (BIophysical ANalysis of Cell death and chromosome Aberrations), was developed [7-9]. BIANCA can compute and predict both chromosome aberrations and cell death, as well as the associated RBE values. The model, implemented as a Monte Carlo code, has only two adjustable parameters, which were calibrated following comparison with survival data for V79 cells, chosen as a reference, exposed to different monochromatic ion beams. This allowed constructing a radiobiological database for V79 cells, consisting of (calculated) alpha and beta cell survival parameters as a function of particle type and energy. An algorithm was then developed to produce analogous databases for other arbitrary cell lines, in a purely predictive way (that is, without further parameter adjustments). Furthermore, BIANCA was interfaced with the FLUKA Monte Carlo radiation transport code. This allowed making RBE predictions for hadron therapy scenarios characterized by complex geometries and mixed fields.

This approach was validated by comparing the BIANCA predictions with *in vitro* survival data for cells other than V79, and with *in vivo* RBE data on the rat spinal cord tolerance following irradiation with protons or carbon ions. Very recently, BIANCA was also applied to the RBE recalculation for two C-ion patient treatment plans, and the outcomes were quantitatively compared with analogous calculations based on LEM and MKM. These validation steps will be illustrated and discussed in this paper.

2. – Materials and methods

2˙1. *The BIANCA Model.* – BIANCA is based on the following, mechanism-based assumptions: i) ionizing radiation can induce DNA "Critical Lesions" (CLs), where by definition each CL produces two independent chromosome fragments; ii) distance-dependent mis-rejoining of these fragments, or fragment un-rejoining, gives rise to chromosome aberrations; iii) certain aberration types (dicentrics, rings and large deletions, where "large" means visible when chromatin is condensed) lead to clonogenic cell death; these aberrations will be called "lethal aberrations". These assumptions, as well as the technical aspects of the simulation procedure, have been discussed in detail in previous works (*e.g.*, [7-9]), and only the main aspects will be reported herein.

Since the features of the critical DNA lesions that lead to chromosome aberrations are still not known in detail (*e.g.*, [10], the model "Critical Lesions" are not defined *a priori*, and their yield (mean number of CLs per Gy and per cell) is an adjustable parameter. Its value mainly depends on radiation quality (*i.e.*, particle type and energy), but is also modulated by the target cell features. The distance dependence of chromosome fragment end-joining is assumed to have the shape of a step function, with threshold distance d equal to the average distance between two adjacent chromosome territories. This function has been shown to be adequate to model the main aspects of clonogenic cell death [11, 12]. Each chromosome fragment is also assumed to have a certain probability, f, to remain un-rejoined even if there are possible partner fragments within the threshold distance. The value of f is the second, and last, adjustable parameter, and is assumed to be cell-line dependent but independent of radiation quality.

A typical simulation with BIANCA requires, as input data, the radiation type (photons, light ions or heavy ions), the model parameters (CL yield and f value), the shape and size of the cell nucleus, and the physical characteristics of the (monochromatic) beam, that is LET (Linear Energy Transfer), energy and absorbed dose. The CLs are uniformly distributed in the cell nucleus for photons, whereas they are located along the particle tracks for ion irradiation. For heavy ions, like carbon, each CL is assumed to have a 0.5 probability to occur at a certain radial distance from the primary track core, to mimic the action of delta rays. For each irradiated cell, the actual number of nucleus traversals and the actual number of CLs per traversal, as well as their positions in the nucleus, are extracted from probability distributions, like the Poisson distribution. Eventually, identification of the hit chromosomes and chromosome arms, simulation of the chromosome-fragment rejoining process, and scoring of the different aberration types are simulated. A cell without any lethal aberration is scored as a surviving cell, otherwise it is counted as a dead cell. Simulation of irradiations at different dose levels provides dose-response curves for both chromosome aberrations and cell death.

2˙2. *Radiobiological databases.* – In previous works [11,12], the model calculations were compared with experimental cell survival curves for V79 cells, considered as a reference due their wide use in radiobiology, including the characterization of hadron therapy beams. In particular, the CL yield was tuned for each ion type (protons, He- and C-ions) and for each LET value, in order to obtain the best agreement between simulations and experimental data. Afterwards, the LET dependence of the CL yield was fitted for each considered ion type, thus allowing to simulate many V79 survival curves over the whole LET range of interest for hadron therapy, using as a code input the CL yield provided by the fit for each considered LET value. Each of these survival curves was then fit by

the familiar Linear-Quadratic (LQ) function:

$$S = e^{-\alpha D - \beta D^2}, \tag{1}$$

and a couple of α and β parameters was then obtained for each LET value. A radiobiological database consisting of these parameter values as a function of particle type and LET was thus created, allowing to perform calculations for every irradiation conditions (for the considered ions and LET ranges) for V79 cells.

However, in view of a possible application of BIANCA in clinical-like scenarios, the model should be able to make predictions for different cell lines, and without any parameter adjustment. An algorithm was thus developed to predict the ion survival of a different cell line starting from the V79 database, as well as the photon response of the cell line of interest. In particular, the CL yield (expressed as CL/μm, that is mean number of CLs per unit traversal length) to be used as an input code to predict the survival of the new cell line for a given radiation quality (that is, ion type and LET), can be derived from

$$\frac{\mathrm{CL}}{\mu\mathrm{m}} = \left(\frac{\mathrm{CL}}{\mu\mathrm{m}}\right)_{\mathrm{ref}} \cdot \left[\left(\frac{\mathrm{CL}}{\mathrm{Gy}\cdot\mathrm{cell}}\right) \Big/ \left(\frac{\mathrm{CL}}{\mathrm{Gy}\cdot\mathrm{cell}}\right)_{\mathrm{ref}}\right] \cdot \frac{V_{\mathrm{ref}}}{V}, \tag{2}$$

where $(\mathrm{CL}/\mu\mathrm{m})_{\mathrm{ref}}$ is the CL yield used to simulate the survival of the reference cell line irradiated with the same radiation quality, $(\mathrm{CL}\cdot\mathrm{Gy}^{-1}\cdot\mathrm{cell}^{-1})$ and $(\mathrm{CL}\cdot\mathrm{Gy}^{-1}\cdot\mathrm{cell}^{-1})_{\mathrm{ref}}$ are the CL yields used to simulate photon irradiation of the cell line of interest and the reference cell line, and V_{ref} and V represent the nucleus volume of the reference cell line and the cell line of interest, respectively. In this way it is now possible to produce a database of α and β parameters for an arbitrary cell line without any further parameter adjustment, and to perform full predictions for the cell line of interest in terms of cell survival and RBE. The latter quantity can be calculated according to the formula [13]

$$\mathrm{RBE} = 2\beta_i[-\alpha_X + \sqrt{\alpha_X^2 - 4\beta_X \ln S}]/2\beta_X[-\alpha_i + \sqrt{\alpha_i^2 - 4\beta_i \ln S}]. \tag{3}$$

Thanks to this method, different databases were produced, including one for CHO (Chinese Hamster Ovary) cells, used for *in vitro* validation of the model, and one for chordoma, used for *in vivo* validation. The photon response of chordoma, which is required to build the database, was deduced from both *in vivo* and *in vitro* data, as described in [13].

2˙3. *Interface with FLUKA.* – In order to make predictions also for complex irradiation geometries and to take into account the presence of mixed fields in realistic hadron therapy scenarios, BIANCA was interfaced with the FLUKA radiation transport code [14-16]. In each voxel of a simulated geometry, FLUKA calculates the necessary physical information, that is particle type and LET and absorbed dose. Afterwards, reading the database provided by BIANCA, FLUKA can associate to each energy deposition the corresponding α and β values. The presence of different particle types in the same voxel is taken into account by means of the approach described in [13], which allows associating to each voxel a mean value of α and β. The corresponding cell survival fraction, RBE-weighted dose (D_{RBE}) and RBE can thus be easily calculated, as illustrated in [12]. In order to reproduce with FLUKA the irradiation geometry in the case of SOBPs, an algorithm to

quickly compute the energies and weights of each particle beam was also developed, as described in [17].

3. – Results and discussion

3˙1. *In vitro predictions*. – The first step for validating the predictive capability of BIANCA consisted of benchmarking it against *in vitro* cell survival data. In the work reported in [11], a first predictive database of α and β parameters was created for U87 glioma cells, for which the experimental photons response was taken from [18]. In the same experimental work, U87 cells were also irradiated at 6 different positions along a SOBP of a therapeutic proton beam available at INFN-LNS in Catania, Italy. Since at that time the interface with FLUKA had not been developed yet, in that work the predictions of BIANCA were performed in the form of monochromatic irradiations with LET values corresponding to the dose-average LET at the six experimental depths, namely 1.1, 4.0, 7.0, 11.9, 18.0 and 22.6 keV/μm. Despite this approximation, the agreement between the predicted cell survival curves and the experimental ones was good, with the only exception of the highest LET.

The same approach was then applied to CHO cells irradiated at HIT by two opposing fields of protons or C-ions mimicking a typical patient treatment scenario, as described in [2]. The photon survival curve was reproduced by BIANCA with a parameter tuning procedure, and a radiobiological database was produced without any further parameter adjustment, following the procedure described in sect. **2**˙2. In this case the 3D irradiation geometry was reproduced with FLUKA, in order to mimic the same physical dose profile of the experimental work. The CHO database produced by BIANCA was then read by FLUKA, thus allowing to calculate the cell surviving fraction in each point of the irradiation geometry. The predictions of BIANCA were found to be in good agreement with the experimental data for both proton and C-ion irradiation, as reported in [12]. Furthermore, a similar experiment for He ions was also performed, for a monodirectional irradiation [2]. This different geometry was also simulated by FLUKA and, following the same approach described above, the BIANCA predictions were found to be in good agreement with the experimental values, as will be described in a future paper.

3˙2. *In vivo predictions*. – In order to validate BIANCA also against *in vivo* data, a database for chordoma cells was produced; this database has been used for all *in vivo* applications performed till now. This approach allowed us to make direct comparisons with the predictions of LEM, which, for historical reasons, was first applied to chordoma and was validated by comparisons with RBE data on the rat spinal cord tolerance. Afterwards, the LEM chordoma table was also used —and is still used— for other tumor types, with few exceptions. In our work, the photon response of chordoma cells was derived from both *in vitro* [19, 20] and *in vivo* [21] data, leading to the following estimation of the LQ parameters: $\alpha_x = 0.159$ and $\beta_x = 0.065$. This photon survival curve was reproduced with BIANCA by tuning the two model parameters, and a predictive radiobiological database for chordoma was produced as described in sect. **2**˙2.

In order to have a preliminary idea of the BIANCA predictions in terms of chordoma RBE, fig. 1 reports the RBE-LET relationship calculated based on the chordoma database for carbon monochromatic irradiation at 1 Gy, which is of interest for the entrance channel of a therapeutic beam, and at 2 Gy, which is of interest for the SOBP. In both cases the predicted RBE shows a steep increase up to a maximum LET value located between

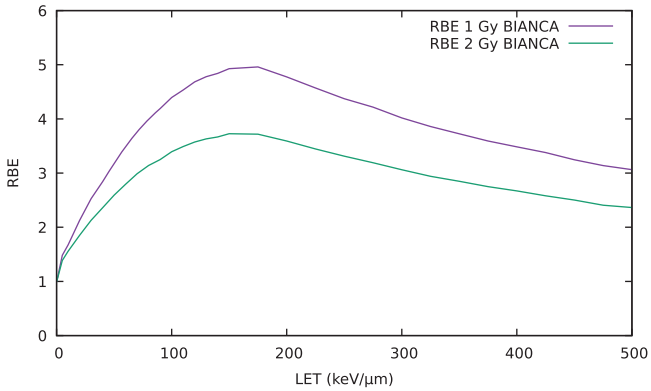

Fig. 1. – RBE-LET relationship calculated by BIANCA for chordoma cell survival following irradiation with different monochromatic carbon beams at 1 Gy (upper curve) or 2 Gy (lower curve) carbon dose.

100 and 200 keV/μm, followed by a decrease in the so-called "overkilling" region. As expected, the RBE at 1 Gy is higher than that at 2 Gy.

The RBE predictions based on the chordoma table were then compared with the same data considered by the LEM authors, who benchmarked their C-ion predictions against *in vivo* data on the rat spinal cord tolerance. In these experiments, the spinal cord was located at six positions of a 6 cm C-ion SOBP (dose-averaged LET values: 16, 21, 36, 45, 66 and 99 keV/μm). The animals were irradiated either by a single fraction [6, 22] or by two fractions [23], and RBE values were calculated by comparison with 15 MV photon

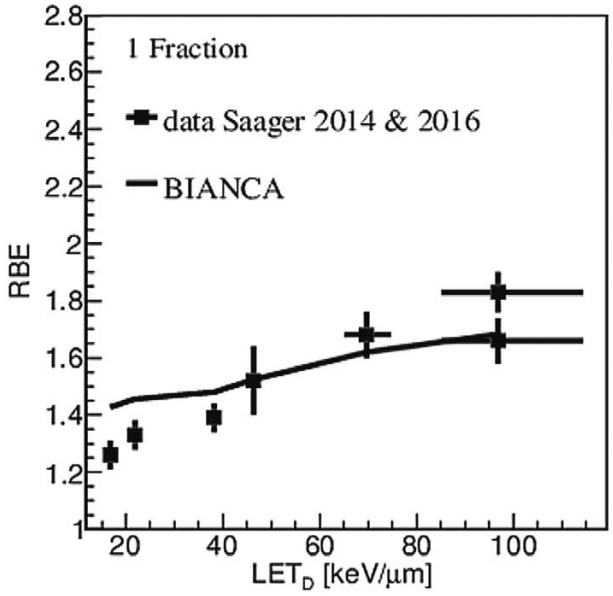

Fig. 2. – RBE as a function of dose-averaged LET for single-fraction irradiation of the rat spinal cord at different positions within a carbon-ion SOBP. The lines are predictions obtained by BIANCA, the points are experimental data taken from [6, 22].

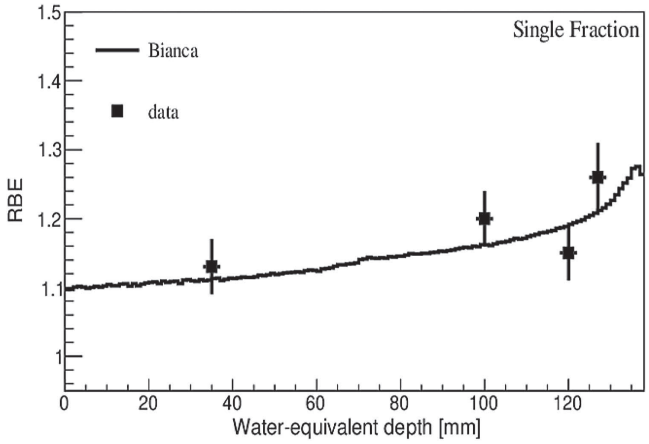

Fig. 3. – RBE as a function of depth for single-fraction proton irradiation of the rat spinal cord. The lines are predictions by BIANCA, the points are experimental data taken from [24].

irradiation. In fig. 2 the predictions of BIANCA are compared with RBE data for the single fraction case as a function of dose-averaged LET. Despite a slight overestimation in the lower LET region, the agreement between data and model predictions is satisfactory. Concerning the overestimation at low LET, it has to be taken into account that the doses considered in these animal experiments were very high (in the range 15–27 Gy). At such high doses, the linear-quadratic survival curves tend to become purely linear, but the BIANCA model does not take into account this behaviour, since it is calibrated against *in vitro* cell survival curves at lower doses. This is true especially at low LET, where the quadratic component at intermediate doses is important, whereas it becomes negligible at higher LET, where the survival curves are almost purely linear. Therefore, BIANCA tends to overestimate cell death at very high doses, for low LET irradiation, and this is reflected also in fig. 2. On the contrary at lower doses, close to the fractional doses used for patient treatment, BIANCA showed a very good agreement with the low-LET data, as reported in [14].

The chordoma database produced by BIANCA was also benchmarked against *in vivo* data for proton irradiation. Figure 3 shows BIANCA predictions compared with RBE data for the rat spinal cord reported in [24]. Analogously to the C-ion experiments, the spinal cord was positioned at four positions (corresponding to dose-averaged LET values of 1.4, 2.7, 3.9 and 5.5 keV/μm) of a 6 cm proton SOBP ranging from 70 to 130 mm, and was irradiated either with a single dose or with two split doses. Only the single fraction case is reported in the figure. The experimental data, which are presented as a function of water-equivalent depth, are well reproduced by the BIANCA predictions, both qualitatively and quantitatively. Overall, these results suggest that BIANCA may be used to predict RBE values for CNS late effects for both C-ions and protons.

3˙3. *Patient cases*. – In a subsequent pilot study, the same chordoma table used to predict the rat spinal cord RBE was applied to two C-ion patient cases, one for chordoma and one for prostate, in order to compare the outputs (in terms of RBE-weighted dose) obtained by BIANCA with those calculated by the LEM I model. Although the results of this study will be published elsewhere [25], the main issues can be summarized as

follows. The RBE-weighted dose calculated by BIANCA for the entrance channels, both for chordoma and for prostate, was significantly lower than that calculated by LEM I. This may be due to the tendency of LEM I to overestimate the RBE at the lower LET values, if doses of few Gy are considered. Concerning the PTV (planned target volume), BIANCA predicted values in line with those of LEM I for chordoma, and slightly lower values for prostate. Finally, BIANCA and LEM I provided similar predictions in the considered organs at risk, which were the brainstem for chordoma and the rectum for prostate [25]. The BIANCA predictions were also compared with those by the MKM model, finding that BIANCA tends to shows a behavior that is intermediate between LEM I and MKM, and thus is compatible with the models currently applied in clinics.

4. – Conclusions

The predictive capability of the BIANCA biophysical model was tested against experimental data on *in vitro* irradiation of U87 cells by monochromatic protons, *in vitro* irradiation of CHO cells along proton, He-ion and C-ion Spread Out Bragg Peaks, and *in vivo* irradiation of rat spinal cord by proton and C-ion SOBPs. The agreement between simulations and experimental data was satisfactory in almost all cases. The model was thus also applied to the RBE prediction for C-ion patient cases (a chordoma and a prostate case), comparing the outcomes with those provided by LEM I and MKM, two models currently applied in clinics. The BIANCA predictions were found to be in line with those of the other two models.

These results are relevant in view of a possible future application of BIANCA for treatment planning biological optimization, since this approach may have some advantages with respect to those that are currently applied. First, while LEM I and MKM are specific for C ions, BIANCA provides good results also for protons and helium, in addition to carbon. This would allow to use a single model for different ion types, and should provide a more reliable description of the secondary-ion biological effectiveness in C-ion treatments. It is worth mentioning that, apart from LEM I and MKM, there are other biophysical models under development, and in particular the updates of LEM, like LEM IV, significantly improved the agreement with experimental data (*e.g.*, [2]). However, in this work we preferred to focus only on the comparison with models that have been applied in clinics so far.

The second advantage is that with BIANCA it would be relatively simple to produce *ad hoc* radiobiological databases for different tumour types starting from their photon response, thus allowing to perform a tumour-specific optimization. Finally, in addition to cell death, BIANCA can also predict chromosome aberrations, some of which (typically, reciprocal translocations) are known to be related to normal tissue (late) damage, typically secondary tumors. As reported in a companion paper [26], it is thus possible to produce radiobiological database(s) for chromosome aberrations. In the future, this would allow to optimize a treatment plan taking into account both the effectiveness at tumor cell killing, and that at inducing normal tissue damage, which would represent a novelty for hadron therapy treatment planning.

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