Modelling the induction of cell death and chromosome damage by therapeutic protons

M.P. Carante $^{1,2}$ and F. Ballarini $^{1,2,*}$

$^1$ University of Pavia, Physics Department, Pavia, Italy
$^2$ INFN, Sezione di Pavia, Pavia, Italy

Abstract
A two-parameter biophysical model called BIANCA (BIophysical ANalysis of Cell death and chromosome Aberrations), which assumes a pivotal role for DNA cluster damage and for “lethal” chromosome aberrations, was applied to calculate cell death and chromosome aberrations for normal and radio-resistant cells along a 62-MeV eye melanoma proton beam. The yield of DNA “Cluster Lesions” and the probability for a chromosome fragment of not being rejoined with any partner were adjustable parameters. In line with other works, the beam effectiveness at inducing both biological endpoints was found to increase with increasing depth, and high levels of damage were found also beyond the dose fall-off, due to the higher biological effectiveness of low-energy protons. This implies that assuming a constant RBE along the whole SOBP, as is currently done in clinical practice, may be sub-optimal, also implying a possible underestimation of normal tissue damage. Furthermore, the calculations suggested that for higher fractional doses, like those delivered in hypo-fractionation regimes, the relative increase in effectiveness along the SOBP may be less pronounced than for lower fractional doses.

1 Introduction
Worldwide, the number of new cases of cancer is expected to increase from 14 million in 2012 to about 22 million over the next two decades [1]. In Europe, cancer is a leading cause of morbidity and mortality, where 3.45 million new cancer cases and 1.75 million deaths from cancer have been estimated for 2012 [2]. About half of the patients diagnosed with cancer undergo radiotherapy. While the most frequently adopted treatment modalities use high energy (~MeV) photon or electron beams, alternative modalities are becoming increasingly important. One example is provided by treatments using proton or carbon ion beams. While the main advantage of carbon ions is a higher biological effectiveness, which makes them particularly suitable to treat those tumours that are resistant to conventional radiotherapy, the rationale of using protons mainly relies on the ability of these particles to reduce the dose to normal tissues, thanks to the dose localization in the (Spread-Out) Bragg peak, or SOBP [3]. In addition to different types of tumors, it is worth mentioning that protons can also be used to treat some non-cancer diseases, such as arteriovenous malformations [4]. According to the Particle Therapy Co-operative Group [5], 49 proton therapy centers were operating and 32 were under construction in June 2015.

Protons are usually considered low-LET radiation, and a constant Relative Biological Effectiveness (RBE) of 1.1 is generally applied in clinics. However, both in vitro and in vivo studies

* Corresponding author (francesca.ballarini@unipv.it)
indicate that proton effectiveness tends to increase with decreasing energy, that is increasing LET [6]. This implies an increase of effectiveness with depth along the SOBP, as well as an extension of the biologically effective range. Furthermore, the RBE depends not only on the particle energy, but also on many other factors including dose, dose-rate, cell type and biological endpoint. For instance, both in vitro and in vivo data show a significant RBE increase for lower fractional doses [7], especially for cells and tissues with low $\alpha/\beta$ ratio. It should also be considered that, although the main endpoint of interest for tumor cells is cell death, other endpoints (e.g. mutations, non-lethal chromosome aberrations...) might be relevant for normal tissues.

Although clinical results do not indicate that the use of a constant RBE is incorrect, one should also consider that no trials specifically targeted RBE variations. Moreover, tighter treatment margins may increase the importance of taking into account such variations [6]. Applying a constant RBE of 1.1 may lead to an underestimation of the damage to normal tissues, especially for treatments involving organs at risk just beyond the tumor, such as the retina for eye tumors. On the other side, the currently available RBE data might be insufficient to support a change in clinical practice [6]. Incorporating variations in biological effectiveness without directly considering the RBE may be an alternative strategy: for instance, it has been suggested that LET distributions in the patient can be used to guide treatment plan optimization [8].

In this framework, a biophysical model of chromosome aberrations [9-11] and cell death [12-15] developed at the University of Pavia and INFN-Pavia, Italy, was applied to AG01522 normal cells and V79 radio-resistant cells exposed at different depth positions along a 62-MeV proton beam used to treat ocular melanoma at INFN-LNS in Catania, Italy [16]. The model, which is called BIANCA (BIophysical ANalysis of Cell death and chromosome Aberrations), assumes that DNA cluster damage can lead to chromosome aberrations and that some aberration types lead to cell death. The fact that the model does not require the use of (experimental) RBE values, which can be a source of uncertainties, represents a potential advantage of this approach. Moreover, the capability of calculating the induction of different types of chromosome aberrations, some of which are related to the risk to normal tissues [17], may be of help for estimating normal tissue damage.

2 The model

The model is based on the following assumptions: 1) radiation induces DNA “Cluster Lesions” (CLs), and each CL produces two independent chromosome fragments; 2) two chromosome fragments can be rejoined only if their initial distance is smaller than a threshold distance $d$, leading to chromosome aberrations in case of rejoining with an incorrect partner; 3) dicentrics, rings and large deletions lead to clonogenic cell death, i.e. the loss of the cell ability to give rise to a colony. Since the characterization of the ‘critical’ DNA damage(s) that can lead to chromosome aberrations and cell death is still an open question in radiobiology, the Cluster Lesions mentioned above were not defined a priori, and the mean number of CLs per Gy and per cell was considered as an adjustable parameter. A previous work [15], in which CL yields for different radiation qualities have been compared with yields of DNA fragments of different sizes, has suggested that clusters of DNA double-strand breaks (DSBs) at the kilo-base-pair scale (which corresponds to geometrical distances in the order of some tens nanometres in the chromatin fibre), possibly in addition to other levels of clustering, may play a relevant role.

Assumption 2) reflects the fact that chromosome fragment rejoining is distance-dependent. In particular, a recent work has suggested the existence in the cell nucleus of “repair centres”, where DSBs should migrate after travelling 1-2 $\mu$m [18]. While in previous works (e.g., [15]) the threshold distance $d$ has been considered as an adjustable parameter, in the present work $d$ was set equal to the mean distance between two adjacent chromosome territories (which resulted to be 3.0 $\mu$m for AG cells and 3.6 $\mu$m for V79 cells, see below), basing on the idea that repair mainly takes place in small
Modelling the induction of cell death and chromosome damage by therapeutic protons

363

channels separating adjacent chromosome domains [19]. According to this approach, \( d \) is fixed \textit{a priori} and depends on the specific features of the considered cell nucleus (i.e., nucleus shape and dimensions and number of chromosomes). This allowed reproducing experimental yields of chromosome aberrations not only for the so-called “lethal aberrations” (i.e. dicentrics plus rings plus deletions), but also for each single aberration category including deletions, which previously were underestimated. While in previous works a chromosome fragment having at least one potential partner for rejoining (that is, at least another fragment within the threshold distance \( d \)) has been assumed to undergo rejoining with 100% probability, in the present work a more realistic scenario was considered where a fragment has a certain probability \( f \) of remaining un-rejoined, even if one or more potential “partners” are available within \( d \). \( f \) was considered as a cell-line-specific parameter, to be adjusted \textit{a posteriori} by comparison with experimental dose-response curves.

Assumption 3) derives from the relationship between chromosome aberrations and cell death shown by many works available in the literature. In particular, for AG1522 normal human fibroblasts exposed to X-rays, Cornforth and Bedford [20] found a one-to-one relationship between the mean number per cell of lethal aberrations and -\( \ln S \), where \( S \) is the fraction of surviving cells. According to another work, an analogous relationship may hold for V79 cells as well [21].

Like in previous works, cell nuclei were modelled as cylinders, with elliptical base for AG cells (major axis: 20 \( \mu m \); minor axis: 10 \( \mu m \)), and circular base for V79 cells (radius: 6 \( \mu m \)). The nucleus thickness was 4 \( \mu m \) for AG cells, 6 \( \mu m \) for V79 cells. A discussion on these choices can be found elsewhere [15]. Each interphase chromosome territory (i.e., the intranuclear region occupied by a chromosome during most of the cell cycle) was represented as the union of adjacent cubic voxels of 0.2 \( \mu m \) side, to obtain chromosome territories with volume proportional to their DNA content. More details on the construction of chromosome territories can be found in [13]. Within the cell nucleus volume, the various CLs were distributed uniformly for X-rays, and along segments parallel to the cylinder axis for (low-energy) protons. A detailed description of this part of the simulation can be found elsewhere [13,14].

The subsequent simulation steps consisted of: identification of the chromosome and the chromosome-arm that was hit by each CL; rejoining of chromosome fragments within the threshold distance \( d \); scoring of dicentrics, rings and large deletions, where “large” means larger than 3 Mbp [20]; calculation of the corresponding surviving fraction. The repetition for different cells (i.e., different runs of the code) provided statistically-significant yields of chromosome aberrations and cell

![Fig. 1](image.png)

**Fig. 1:** Relative dose (asterisks) and relative fraction of inactivated cells for AG01522 (full circles) and V79 (empty circles) cells at different depth positions. The lines are simply guides for the eye.
death after a given dose, and the repetition for different doses provided simulated dose-response curves directly comparable with experimental data.

3 Calculation of cell death and chromosome aberrations for a eye-melanoma proton beam

Following the reproduction of cell survival curves in previous works [13-15], in the present work the model was applied to investigate the depth- and dose-dependence of the Catania beam effectiveness, both in terms of cell death and in terms of chromosome aberrations. For different depths of the proton SOBP dose profile reported in [16], figure 1 reports calculated relative fractions of inactivated cells assuming a dose of 2 Gy in the plateau region, together with the relative dose. “Relative” means that the various quantities were normalized with respect to the proximal position. With respect to the experimental work considered for comparison, the simulations allowed predicting AG01522 cell death also for additional positions, with particular attention on the dose fall-off region that can be critical for normal tissue damage. Furthermore, the model allowed predicting cell death also for V79 cells, which have not been investigated in the experiments.

Consistent with the experimental data reported in [16] and with other works available in the literature (e.g., [7]), the beam effectiveness was found to increase with depth along the plateau, and high levels of cell death were found also beyond the dose fall-off. For instance at ~31 mm, where the physical dose was about 40% of the proximal dose, the fraction of AG01522 inactivated cells was almost 80% with respect to the proximal position. This can be explained taking into account that, as protons slow down, their LET increases, leading to a higher biological effectiveness. Interestingly, the increase in biological effectiveness was different for the two considered cell types: while AG01522 cells tended to show a continuous increase along the whole plateau, for V79 cells the effectiveness remained basically flat for most of the plateau, but increased sharply in the distal region. This kind of behavior for V79 cells is consistent with the characteristics of this cell line – and, more generally, cells
with a low $\alpha/\beta$ ratio, which is rather radio-resistant at low LET but tends to become particularly sensitive to LET variations when the LET increases.

Figure 2 reports predicted relative yields of chromosome aberrations for different depth positions along the same dose profile, again assuming a dose of 2 Gy in the plateau region; the relative dose already shown in figure 1 is reported as well. Among the various chromosome aberration types, the attention was focused on dicentrics, since dicentric yields are considered as representative of the yields of reciprocal translocations, which can be related to cell conversion to malignancy [17] and thus can help evaluating the damage to normal tissues.

Like for cell death, also for chromosome aberrations the beam effectiveness increased with depth along the plateau, and high aberration yields were found also beyond the dose fall-off. Moreover, AG01522 cells showed a continuous increase in the yields of dicentrics along the whole plateau, whereas for V79 cells the dicentric yield remained flat along most of the plateau, and showed a sharp increase in the distal region. For both cell types, the increase in chromosome aberrations with depth was more pronounced than the increase in cell killing: dicentrics in the distal region were higher by a factor $\sim 1.5$ with respect to the proximal region for both cell types, whereas cell death increased by a factor that was less than 1.3 for V79 cells, and less than 1.1 for AG01522 cells. This is consistent with many experimental observations, and is a typical example of how the beam effectiveness can depend on the considered biological endpoint.

Predictions of cell death and chromosome aberrations were also performed assuming different plateau doses, which can be interesting for hyper- or hypo-fractionation regimes. Figures 3 and 4 report the relative fraction of inactivated cells and the relative mean number of dicentrics per cell for AG01522 and V79 cells, respectively, calculated assuming a plateau dose of 4 Gy (full symbols). For comparison, the corresponding results for 2 Gy (empty symbols) are also shown. For both endpoints and both cell lines, increasing the physical dose from 2 to 4 Gy in the plateau reduced the increase in biological effectiveness along the plateau itself. This is consistent with the well known dose-dependence of RBE, which tends to become lower at higher doses and vice-versa. However, for V79
366     F. Ballarini, M.P. Carante

This effect was more pronounced than for AG01522 cells. This can be explained taking into account that V79 photon survival curves are characterized by a lower $\alpha/\beta$ ratio and thus a more pronounced “shoulder”, which implies a higher variation of effectiveness with dose. Concerning the fall-off region, a peculiar behavior, inverse with respect to the plateau region, was found for cell death in AG01522 cells, for which the increase in effectiveness at inducing cell death was more pronounced at the higher doses with respect to the lower doses. This issue, which is under investigation, might be related to the small shoulder that characterizes AG01522 photon survival curves.

4 Conclusions

A two-parameter biophysical model called BIANCA, which assumes a pivotal role for DNA cluster damage and for “lethal” chromosome aberrations, was applied to calculate cell death and chromosome aberrations for normal and radio-resistant cells along a 62-MeV eye melanoma proton beam. In line with other works, the beam effectiveness at inducing both biological endpoints was found to increase with increasing depth and high levels of damage were found also beyond the dose fall-off, due to the higher biological effectiveness of low-energy protons. This implies that assuming a constant RBE along the whole SOBP, as is currently done in clinical practice, may be sub-optimal, also implying a possible underestimation of normal tissue damage. Furthermore, the calculations suggested that for higher fractional doses, like those delivered in hypo-fractionation regimes, the increase in effectiveness may be less pronounced. More generally, considering the uncertainties that affect the currently available RBE data, this work may be regarded as a starting basis for future characterizations of therapeutic hadron beams without making use of RBE. Of course, before becoming of practical use, the model/code needs to be further refined (e.g., extending it to other cell lines) and “coupled” to a radiation transport code and/or a Treatment Planning System.

Acknowledgements

The authors wish to thank K. Prise and G. Schettino for useful discussions.
References