

# COMPARISON OF RADIOBIOLOGICAL EFFECTS OF CARBON IONS TO PROTONS ON A RESISTANT HUMAN MELANOMA CELL LINE

I. Petrović<sup>a</sup>, A. Ristić-Fira<sup>a</sup>, L. Korićanac<sup>a</sup>, J. Požega<sup>a</sup>,  
F. Di Rosa<sup>b</sup>, P. Cirrone<sup>b</sup> and G. Cuttone<sup>b</sup>

*a) Vinča Institute of Nuclear Sciences, Belgrade, Serbia*

*b) INFN Laboratori Nazionali del Sud, Catania, Italy*

- § High ionizing radiation has been commonly used in radiotherapy because of its good radiobiological response in killing malignant growths.
- § A number of studies have investigated the effects of protons and other heavier charged particles on various cell types, most of them being quite radiosensitive.
- § Almost no studies have been done regarding behavior of rather radioresistant cell lines.
- § Present results are a part of an extensive study analyzing radiobiological parameters and viability levels of a resistant melanoma cell line after exposure to conventional and different high ionizing radiation.

# Goal

- § Evaluation of physical and radiobiological parameters of the CATANA (Centro di Adro Terapia e Applicazioni Nucleari Avanzati) proton beam facility, used for the treatment of eye melanoma.
- § Assessment of the effects of two types of proton beams (at the Bragg peak maximum and along the spread-out Bragg peak - SOBP) and a  $^{12}\text{C}$  ion beam (along the Bragg curve), on a resistant human HTB140 melanoma cell line, needed to analyse and predict success of therapeutic irradiations for the limit case.
- § Parameters of analyses:
  - level of cell inactivation,
  - quality of cell inactivation.

# Cell culture conditions

- § Irradiation of exponentially growing HTB140 human melanoma cells,
- § Plating efficiency (PE) for HTB140 cells - approximately 70%,
- § Doubling time (Td) for HTB140 cells -  $24 \pm 2,7$  h.

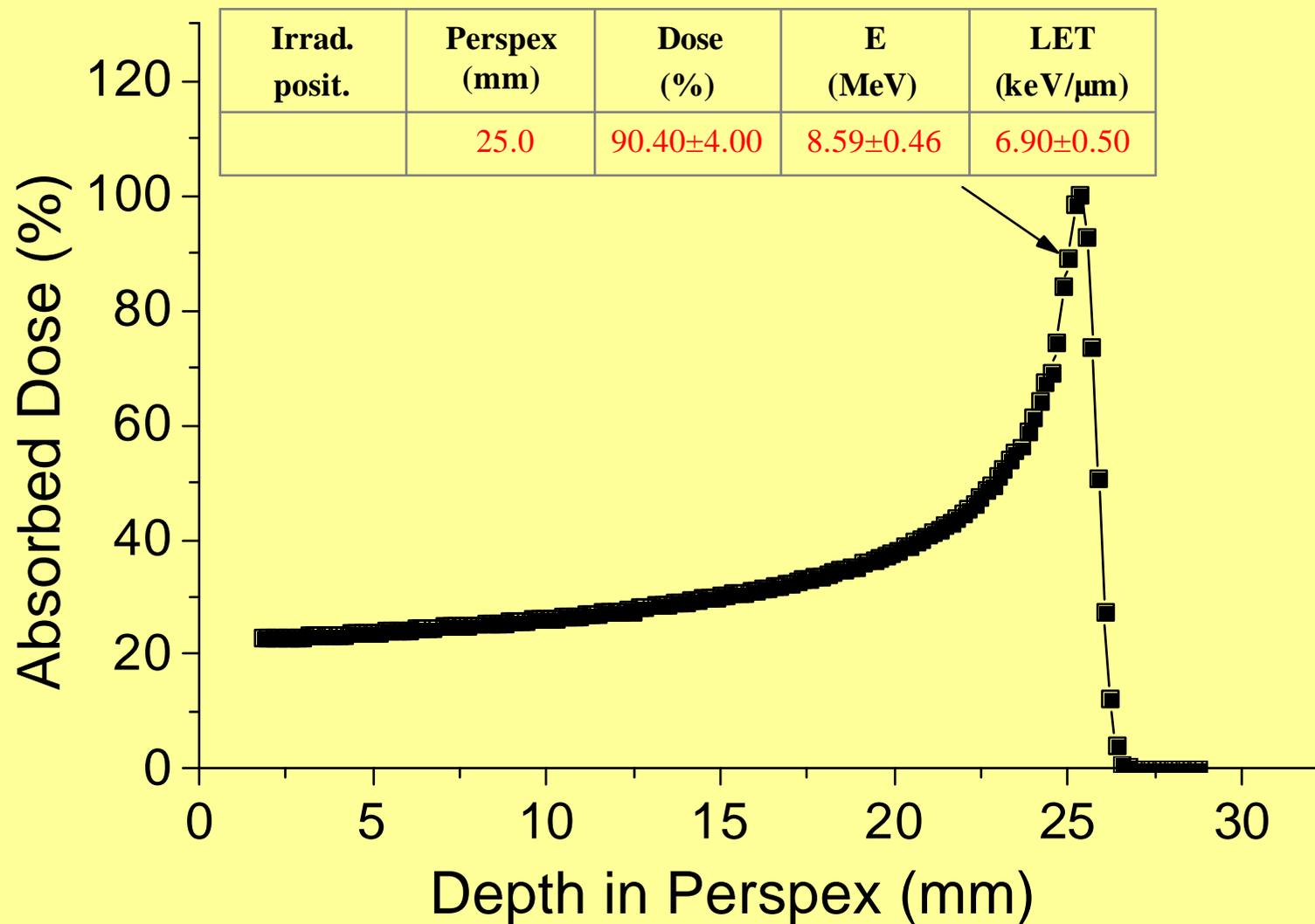
# Irradiation conditions

- § Irradiations with 62 MeV/u (superconducting cyclotron at the CATANA treatment facility, INFN, LNS – Catania):
  - protons,
  - proton spread out Bragg peak (SOBP),
  - $^{12}\text{C}$  ions.
- § Irradiation positions were obtained by interposing Perspex plates (PMMA) of different thicknesses in front of cells.
- § Reference dosimetry - plane-parallel PTW 34045 Markus ionization chamber calibrated according to IAEA code of practice (IAEA-TRS-398 2000).
- § Single doses delivered to the cells ranged from 2 to 24 Gy, dose rate - 15 Gy/min.
- § Reference irradiations with  $^{60}\text{Co}$   $\gamma$ -rays, at the same dose levels, at average dose rate - 1 Gy/min (Vinča Institute of Nuclear Sciences – Belgrade).
- § Particle mean energy ( $\bar{E}$ ) and linear energy transfer (LET) values were obtained by simulations with GEANT4 code.

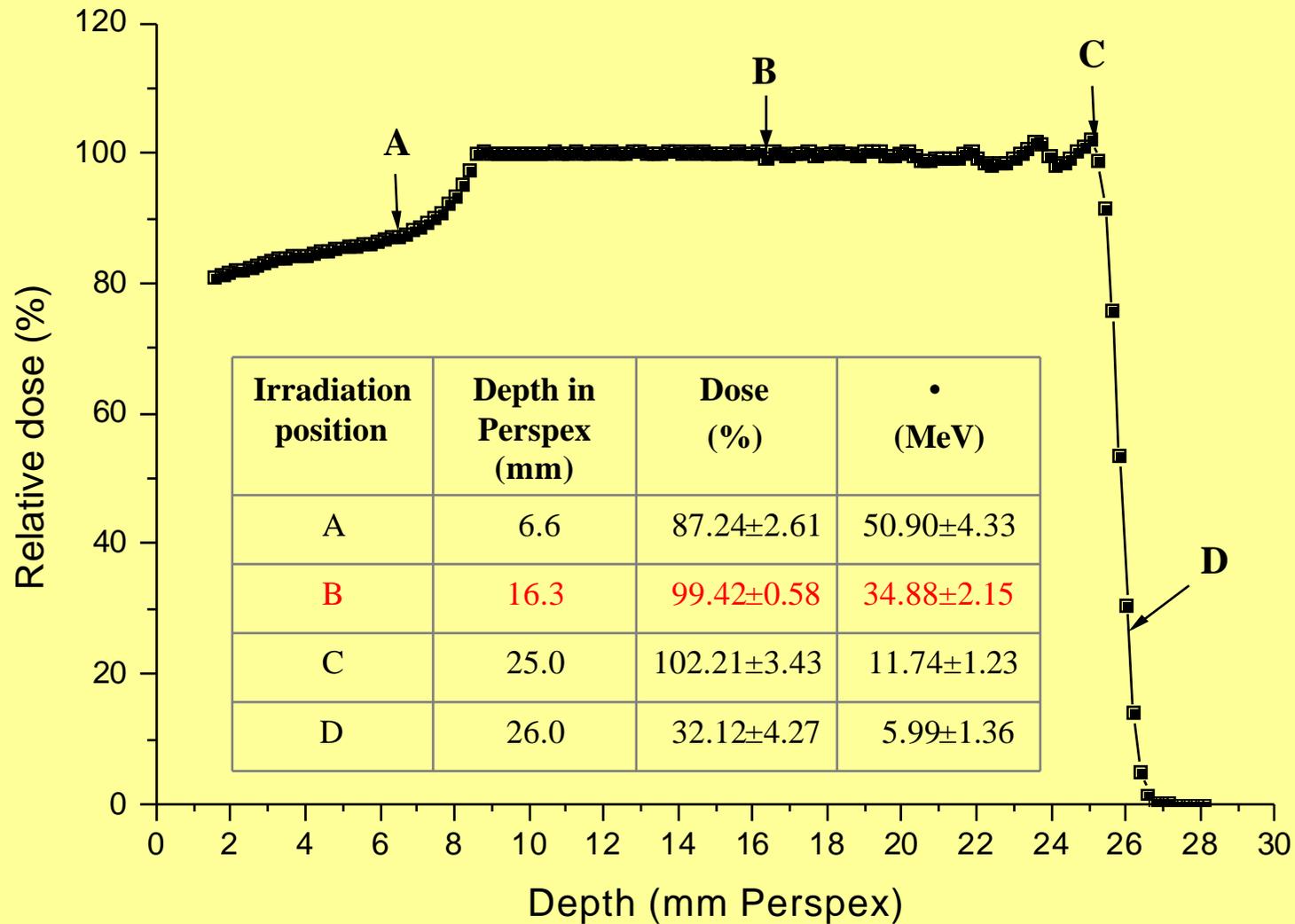
The CATANA (Centro di Adro Terapia e Applicazioni Nucleare Avanzati) treatment facility, INFN - LNS, Catania, Italy.



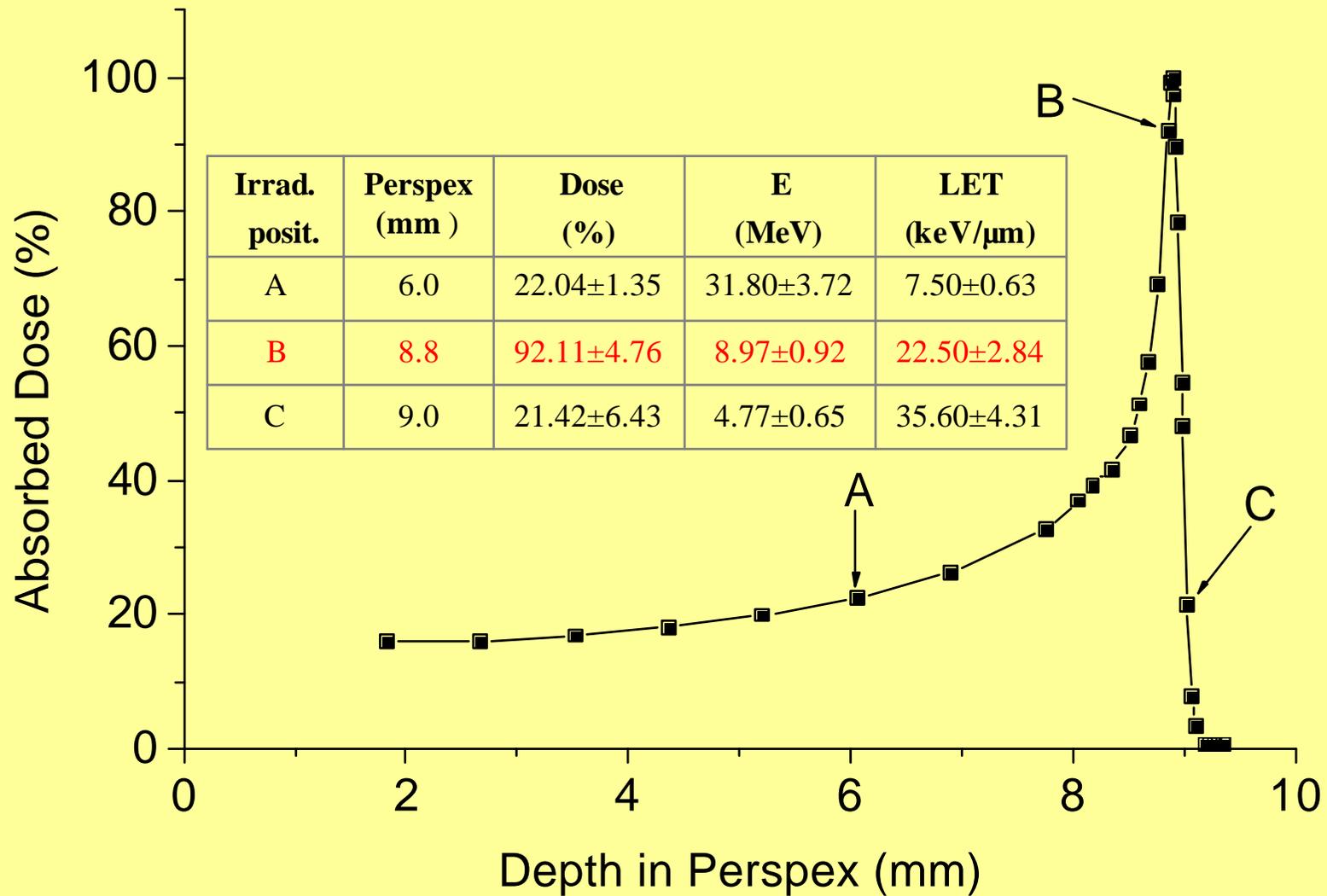
# Depth dose distribution in Perspex of the 62 MeV proton beam.



# Depth dose distribution of the spread out Bragg peak in Perspex of the 62 MeV proton beam.



# Depth dose distribution in Perspex of the 62MeV/u $^{12}\text{C}$ ion beam.



# Biological assays

## § Cell viability:

- clonogenic assay (CA).

## § Cell proliferation:

- incorporation of 5-bromo-2'-deoxyuridine (BrdU) during DNA synthesis.

# Radiobiological parameters

§ Surviving fraction (SF2),

§ Relative biological effectiveness (RBE) - inactivation capacity of irradiated cells.

RBE(2Gy,  $\gamma$ ) is the ratio of 2 Gy  $\gamma$ -ray dose and the proton dose generating the same inactivation level as that given by 2 Gy of the reference  $\gamma$ -rays.

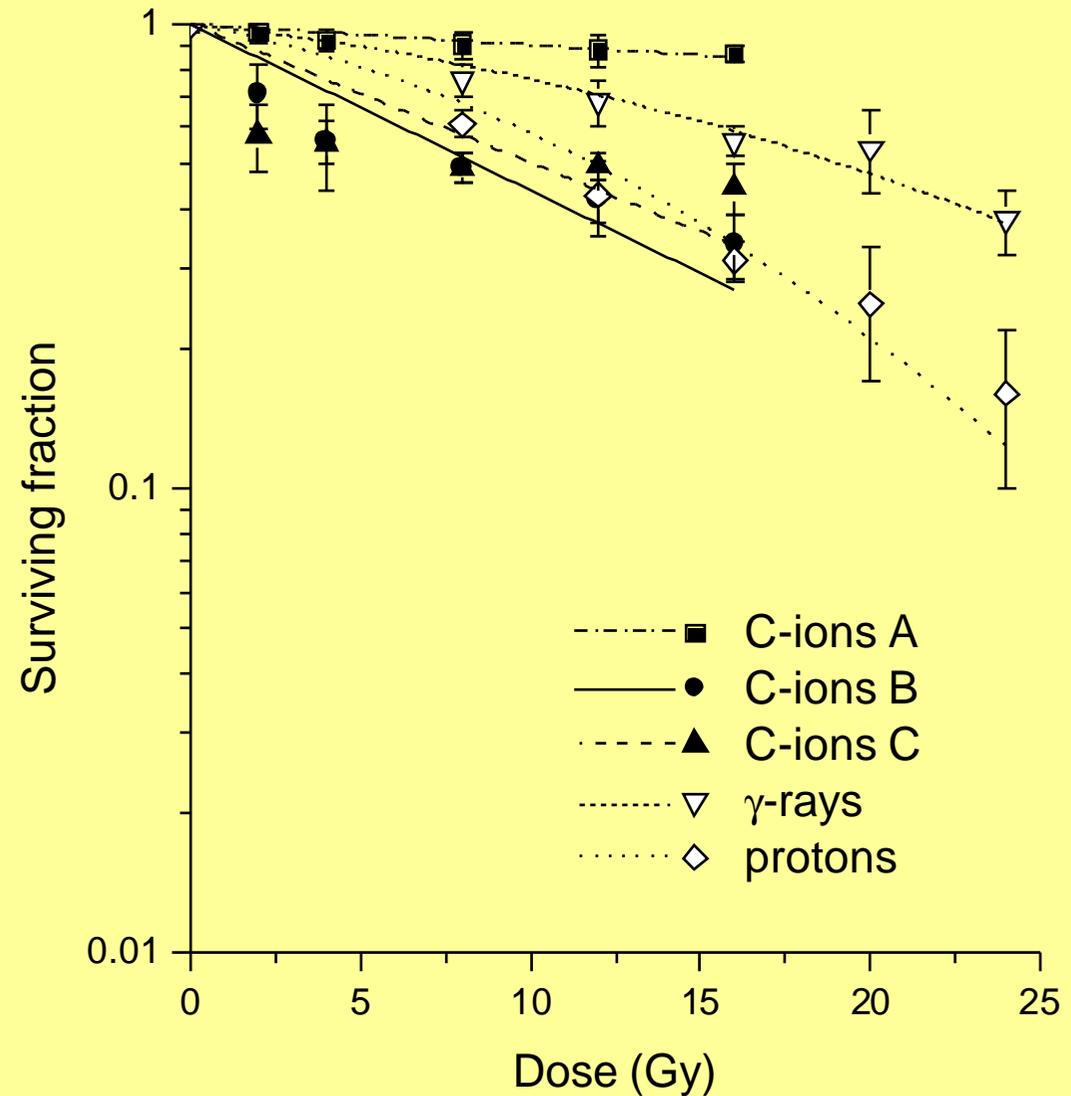
Survival curves – best fit by the linear quadratic expression:

$$S = \exp(-\alpha \cdot D - \beta \cdot D^2),$$

*S* - surviving fraction having dose **D**,

$\alpha$  and  $\beta$  - free parameters.

Dose dependent survival curves after irradiation with  $^{12}\text{C}$  ions (positions A, B and C), protons and  $\bullet$ -rays.

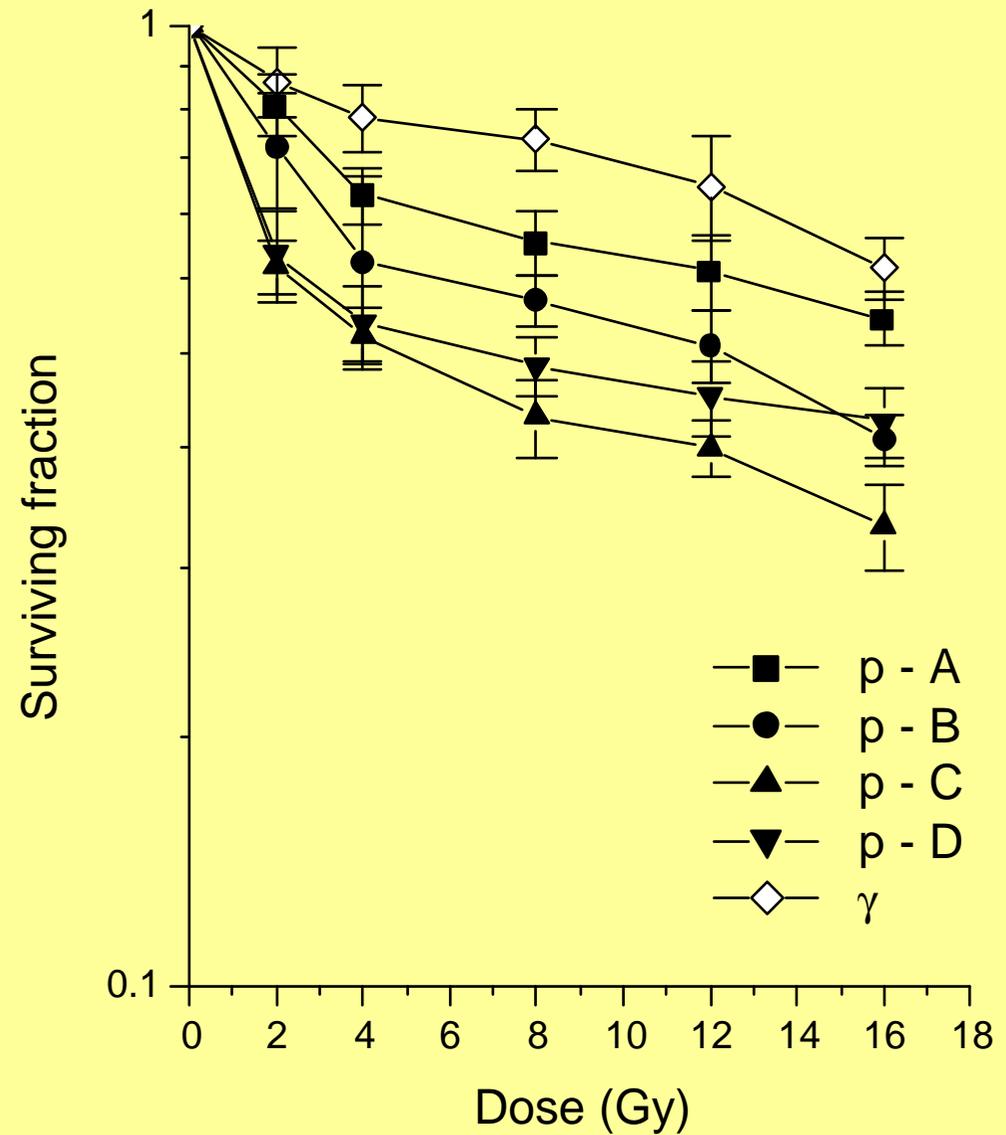


- § Close to the end of range fluence falls down very fast to zero, while LET rises exponentially, producing the distal declining end of the Bragg curve.
- § Consequently, the decreasing number of particles has fewer hits on irradiated cells. However, these hits by particles with increasing LET cause more irreparable and less reparable lesions.
- § Therefore, there is a relatively higher killing ability of a smaller number of particles when approaching the end of range. This explains why dose dependent survival curves for  $^{12}\text{C}$  ions at irradiation positions B and C are close although there is an important difference in dose levels.

§ Stronger killing ability of  $^{12}\text{C}$  ions compared to protons is the consequence of their higher LET (22.50 keV/• m vs. 8.97 keV/• m, respectively), while particles mean energy of  $^{12}\text{C}$  ions and protons are quite close (8.97 MeV and 8.59 MeV, respectively).

§ Track structure of the energy deposition, including fragmentation and secondary particles, plays an important role and has to be taken into account when analyzing biological responses to different ion species. Therefore different biological responses are caused by the different track structure for different atomic numbers with similar LET values.

Dose dependent  
surviving fractions after  
irradiation with protons  
(SOBP positions A, B,  
C and D) and  $\gamma$ -rays.



# Radiobiological parameters for different types of radiations

| Irrad.                 | Irrad. position | SF(2 Gy)           | RBE(2 Gy, •)     |
|------------------------|-----------------|--------------------|------------------|
| <b>proton<br/>SOBP</b> | A               | 0.825±0.061        | 1.39±0.06        |
|                        | <b>B</b>        | <b>0.748±0.103</b> | <b>2.14±0.11</b> |
|                        | C               | 0.562±0.036        | 4.63±0.23        |
|                        | D               | 0.578±0.064        | 4.26±0.28        |

| Irrad.                | Irrad. position | SF(2 Gy)           | RBE(2 Gy, •)     |
|-----------------------|-----------------|--------------------|------------------|
| <b><sup>12</sup>C</b> | A               | 0.979 ±0.001       | -                |
|                       | <b>B</b>        | <b>0.706±0.114</b> | <b>3.38±0.64</b> |
|                       | C               | 0.576 ±0.092       | 3.29 ±0.58       |

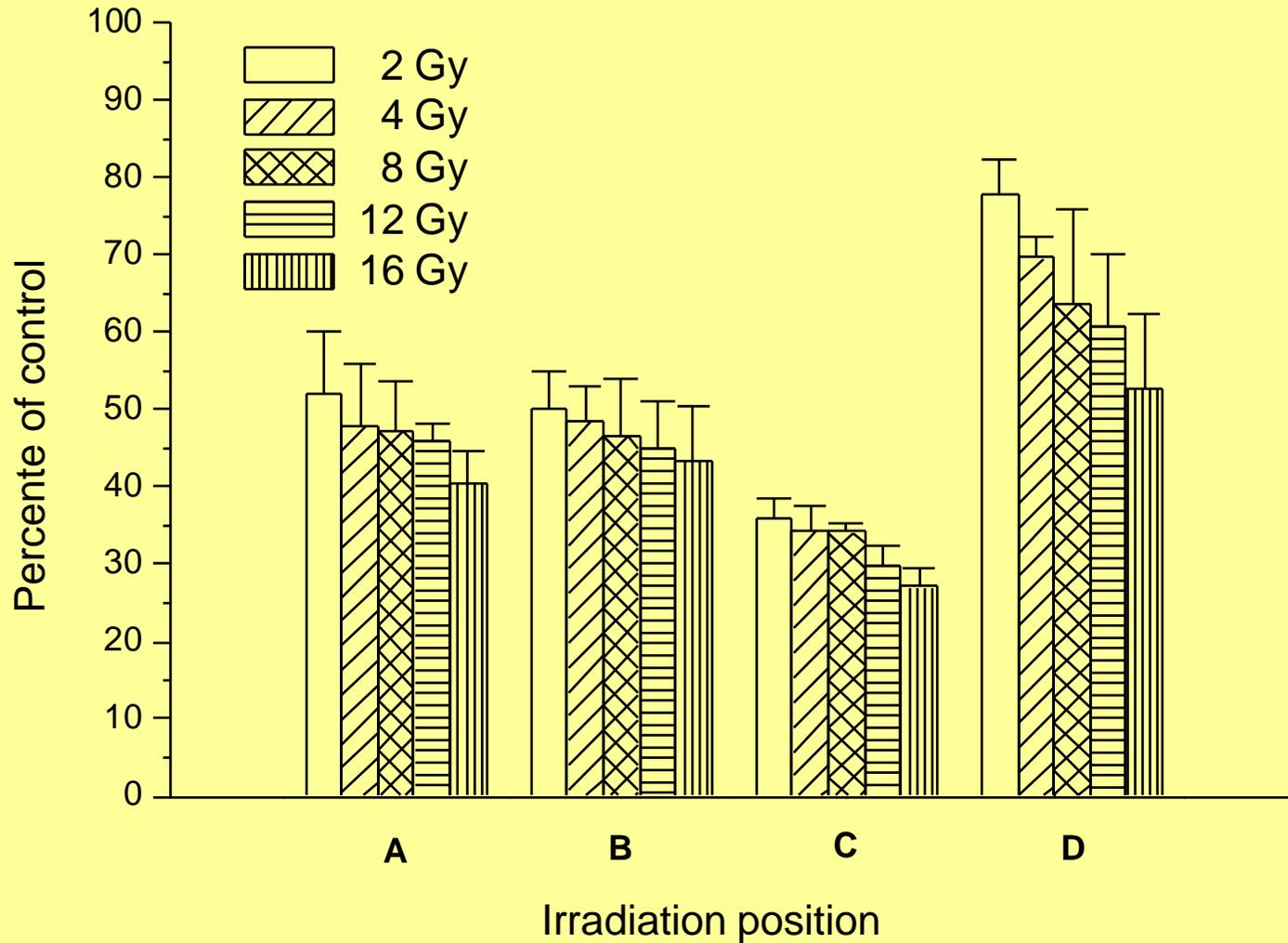
| Irradiation                | SF(2 Gy)    | RBE(2 Gy, •) | RBE(8 Gy, •) | RBE(12 Gy, •) | RBE(16 Gy, •) |
|----------------------------|-------------|--------------|--------------|---------------|---------------|
| <b>•-rays</b>              | 0.961±0.004 | 1            | 1            | 1             | 1             |
| <b>protons</b>             | 0.931±0.006 | 1.69±0.17    | 1.61±0.09    | 1.64±0.14     | 1.63±0.13     |
| <b>mid SOBP</b>            | 0.748±0.103 | 2.14±0.11    | 3.16±0.87    | 3.26±0.79     | 2.89±0.59     |
| <b><sup>12</sup>C-ions</b> | 0.706±0.114 | 3.38±0.64    | 3.30±0.39    | 2.80±0.42     | 2.47±0.34     |

§ Surviving fractions at 2 Gy (SF(2Gy)) are rather high for all irradiations applied, decreasing towards  $^{12}\text{C}$  ions.

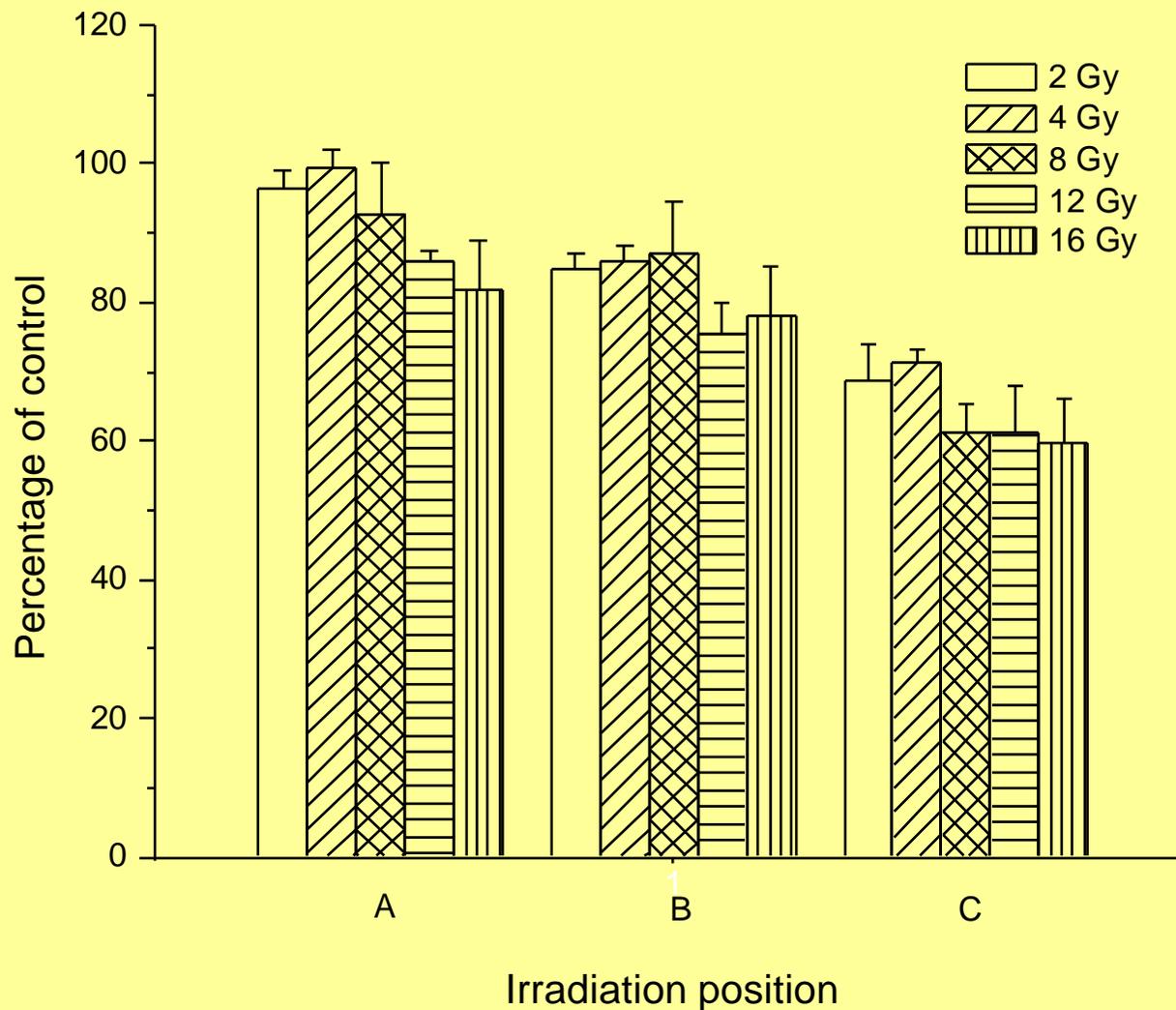
§ Relative biological effectiveness (RBE) with respect to  $\bullet$ -rays at 2 Gy, increases towards  $^{12}\text{C}$  ions. Irregular dose dependence of RBE both for  $^{12}\text{C}$  ions and mid of proton SOBP is caused by different fitting precision.

§ In spite of such high effectiveness of applied irradiation types, these cells remain highly radioresistant with quite low cell inactivation level.

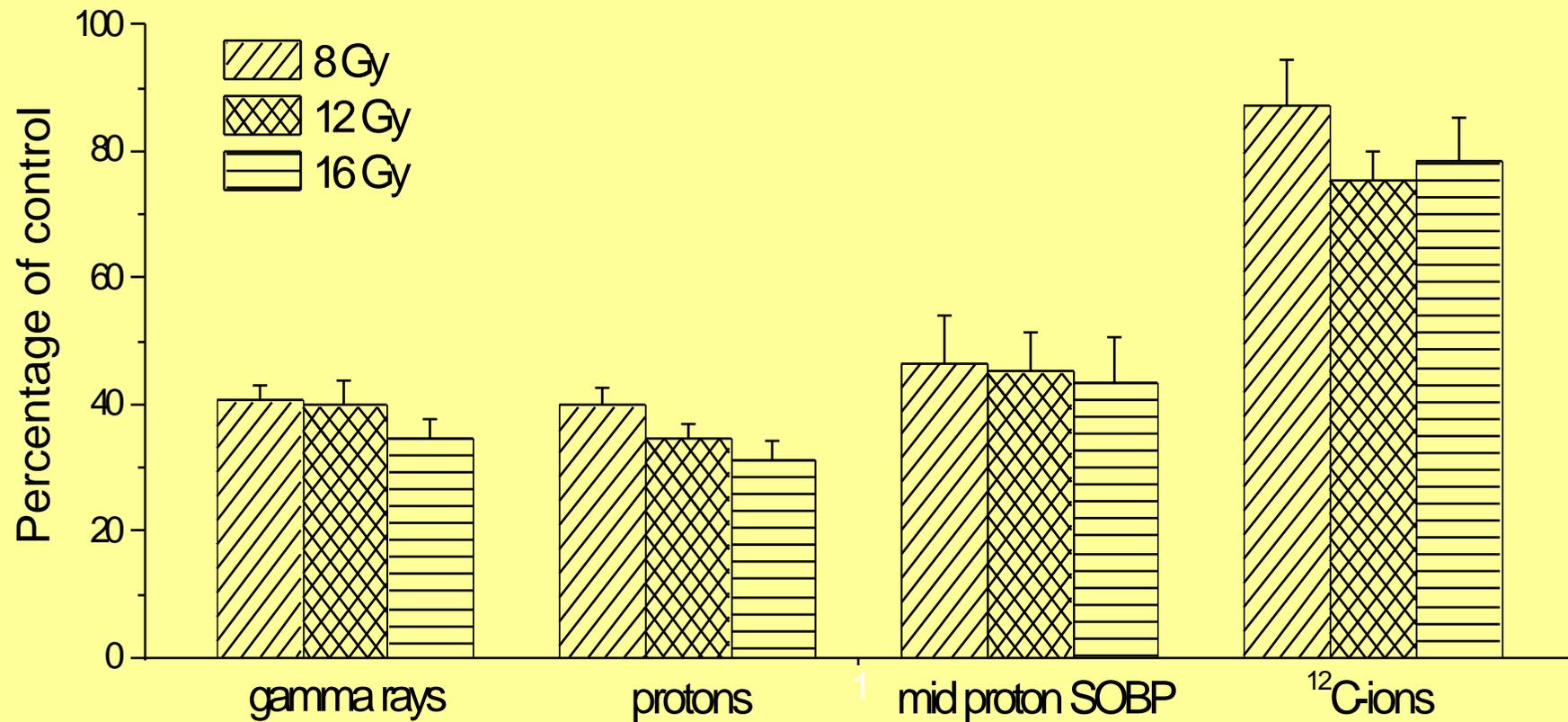
# Dose dependant cell proliferation as a function of depth within the proton spread-out Bragg peak, estimated by BrdU assay.



# Dose dependant cell proliferation as a function of depth within $^{12}\text{C}$ ion Bragg curve, estimated by BrdU assay.



# Comparison of proliferation capacities after exposure to different types of irradiation, estimated by BrdU.



- § Proliferation capacity, monitored through ability of irradiated cells to incorporate BrdU, indicates that  $\bullet$ -rays and unmodulated protons produce, apart from irreparable damages, as shown by corresponding survival curves, also reparable damage to exposed cells, therefore provoking cell cycle arrest and relatively low DNA duplication.
- § Irradiations in the middle of the proton SOBP, because of contributions of higher LET components of superimposed Bragg curves, and particularly close to the Bragg peak maximum of  $^{12}\text{C}$  ions, due to their higher LET, reveal that more irreparable, but less reparable lesions, are induced. This leads to higher level of replication.

Another important concern is the effect of particles at the distal fall off part of the Bragg curve. This is particularly important for hadron therapy planning when a vital healthy tissue is located just behind the treated tumour.

Proliferation capacity estimated after irradiation with  $^{12}\text{C}$  ions (position C) and protons (distal declining end of SOBP).



- § Relatively high values of dose dependant cell proliferation at the distal declining end of proton SOBP and  $^{12}\text{C}$  ions Bragg curve are due to a smaller number of hits to irradiated cells and to a larger number of irreparable lesions induced by these hits. Thus a greater number of cells can maintain active proliferation instead of being involved in complex repair processes.
- § Cell proliferation capacities at the irradiation positions at the distal declining end of the proton SOBP and of  $^{12}\text{C}$  ions (with dose levels of  $32.12 \pm 4.27$  % and  $21.42 \pm 6.43$  %, respectively) are quite close, implying similar levels of irreparable and reparable lesions.
- § Considering that the difference in dose levels is about 10 % and that the degrees of cell proliferation are almost equal, one might expect that for the same dose levels  $^{12}\text{C}$  ions would, due to their higher LET, produce more irreparable and less reparable damages to the cells affected by irradiation, thus reaching higher proliferation level.

# CONCLUSION

- § After irradiations with  $\bullet$ -rays, protons and  $^{12}\text{C}$  ions evaluated radiobiological parameters have shown high resistance of human HTB140 melanoma cells. Due to higher LET,  $^{12}\text{C}$  ions have caused better effectiveness compared to protons.
- § When receiving maximum dose, cells irradiated with  $\bullet$ -rays or protons, having lower LET, have shown lesser dose dependent proliferation than for  $^{12}\text{C}$  ions. This is correlated to different quality of damage produced in irradiated cells.
- § There is a relatively higher inactivation capacity of protons and  $^{12}\text{C}$  ions on the distal declining end of the Bragg curve compared to the Bragg peak.

*Reported study, dedicated to the limit of cellular radiosensitivity, reveals how far protons and  $^{12}\text{C}$  ions could reach in producing biological response.*