USE OF GEANT4 CODE FOR VALIDATION OF RADIOBIOLOGICAL PARAMETERS OBTAINED AFTER PROTON AND CARBON IRRADIATIONS OF MELANOMA CELLS

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Experimental goal

- Present experimental 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.

- To analyse and predict success of therapeutic irradiations for the limit case assessment of the effects of two types of proton beams (at the Bragg peak maximum and along the spread-out Bragg peak - SOBP) and of a ^{12}C ion beam (along the Bragg curve) on the HTB140 human melanoma cells was performed.

- Parameters of the analyses:
  - level of cell inactivation,
  - quality of cell inactivation.

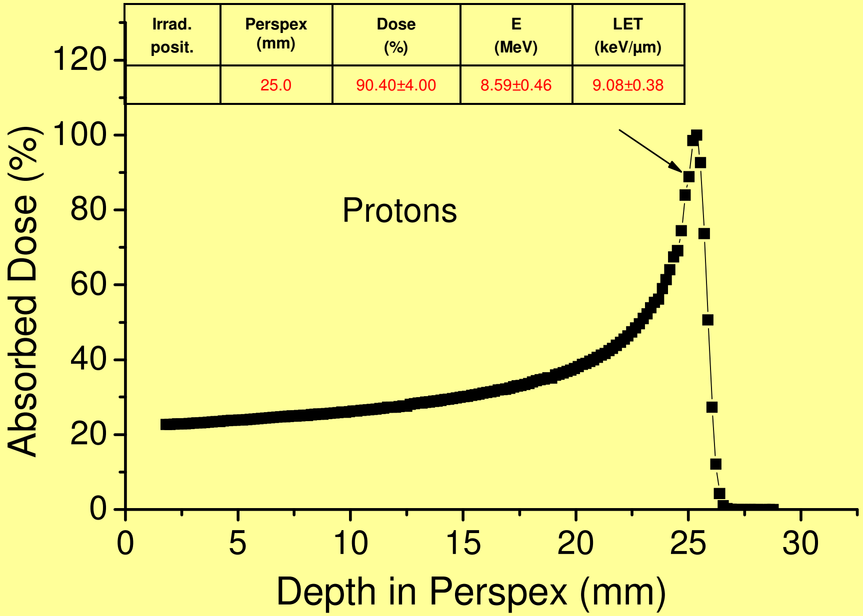
Simulation goal

- Series of numerical simulations using the GEANT4 code were carried out to verify and better interpret radiobiological parameters and biological effects obtained after irradiation of the HTB140 human melanoma cells.

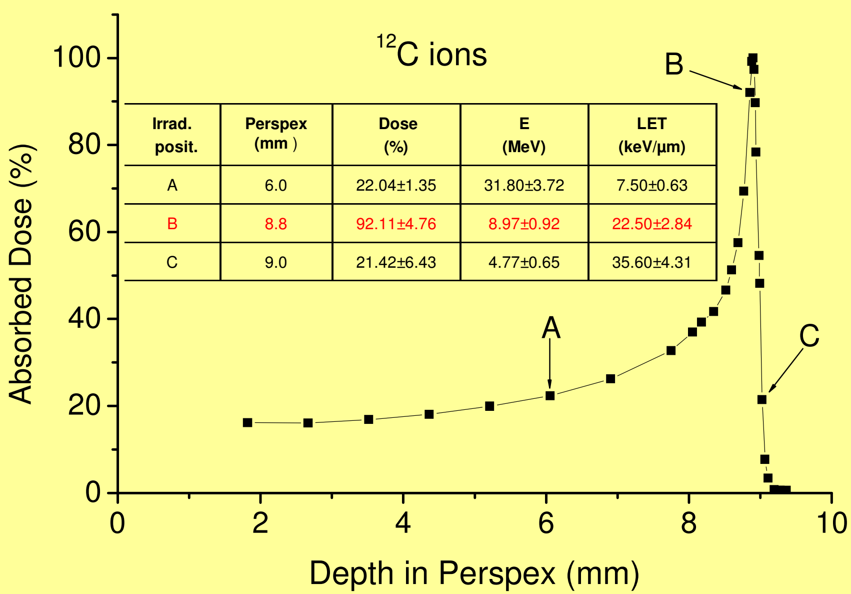
- Parameters of the analyses:
    - dose,
    - energy,
    - fluence,
    - linear energy transfer (LET).

Irradiation conditions

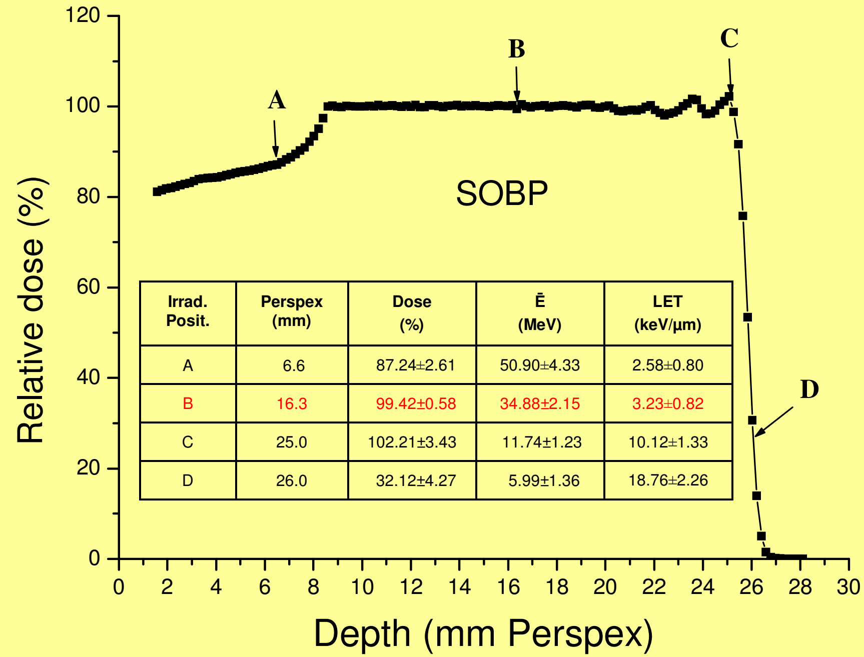
- Irradiations with 62 MeV/u (superconducting cyclotron at the CATANA treatment facility, INFN, LNS – Catania):
    - protons,
    - proton spread out Bragg peak (SOBP),
    - ^{12}C ions.
- Irradiation positions were obtained by interposing Perspex plates (PMMA) of different thicknesses in front of 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}Co γ-rays were, at the same dose levels, at average dose rate - 1 Gy/min (Vinča Institute of Nuclear Sciences – Belgrade).
- Particle mean energy (Ē), linear energy transfer (LET), fluence and dose vales were simulated by GEANT4 code.



Irrad. posit.	Perspex (mm)	Dose (%)	E (MeV)	LET (keV/µm)
	25.0	90.40±4.00	8.59±0.46	9.08±0.38



Irrad. posit.	Perspex (mm)	Dose (%)	E (MeV)	LET (keV/µm)
A	6.0	22.04±1.35	31.80±3.72	7.50±0.63
B	8.8	92.11±4.76	8.97±0.92	22.50±2.84
C	9.0	21.42±6.43	4.77±0.65	35.60±4.31



Irrad. Posit.	Perspex (mm)	Dose (%)	Ē (MeV)	LET (keV/µm)
A	6.6	87.24±2.61	50.90±4.33	2.58±0.80
B	16.3	99.42±0.58	34.88±2.15	3.23±0.82
C	25.0	102.21±3.43	11.74±1.23	10.12±1.33
D	26.0	32.12±4.27	5.99±1.36	18.76±2.26

Biological assays

- Cell viability:
  - clonogenic assay (CA).

- Cell proliferation:
  - incorporation of 5-bromo-2`-deoxyuridine (BrdU) during DNA synthesis.

Radiobiological parameters

- Surviving fraction at 2 Gy (SF2),
- Relative biological effectiveness (RBE) - inactivation capacity of irradiated cells.

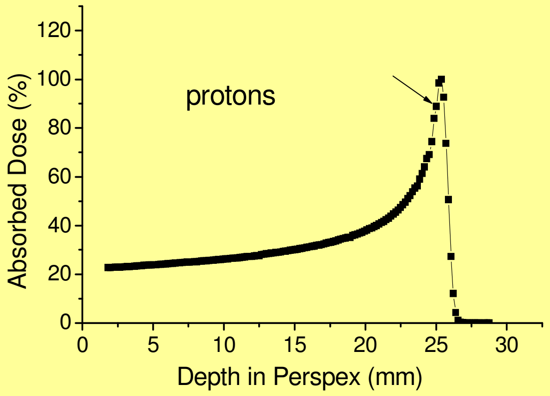
RBE(2Gy, γ) is the ratio of 2 Gy γ–ray dose and the proton dose generating the same inactivation level as that given by 2 Gy of the reference γ–rays.

Survival curves – best fit by the linear quadratic expression:

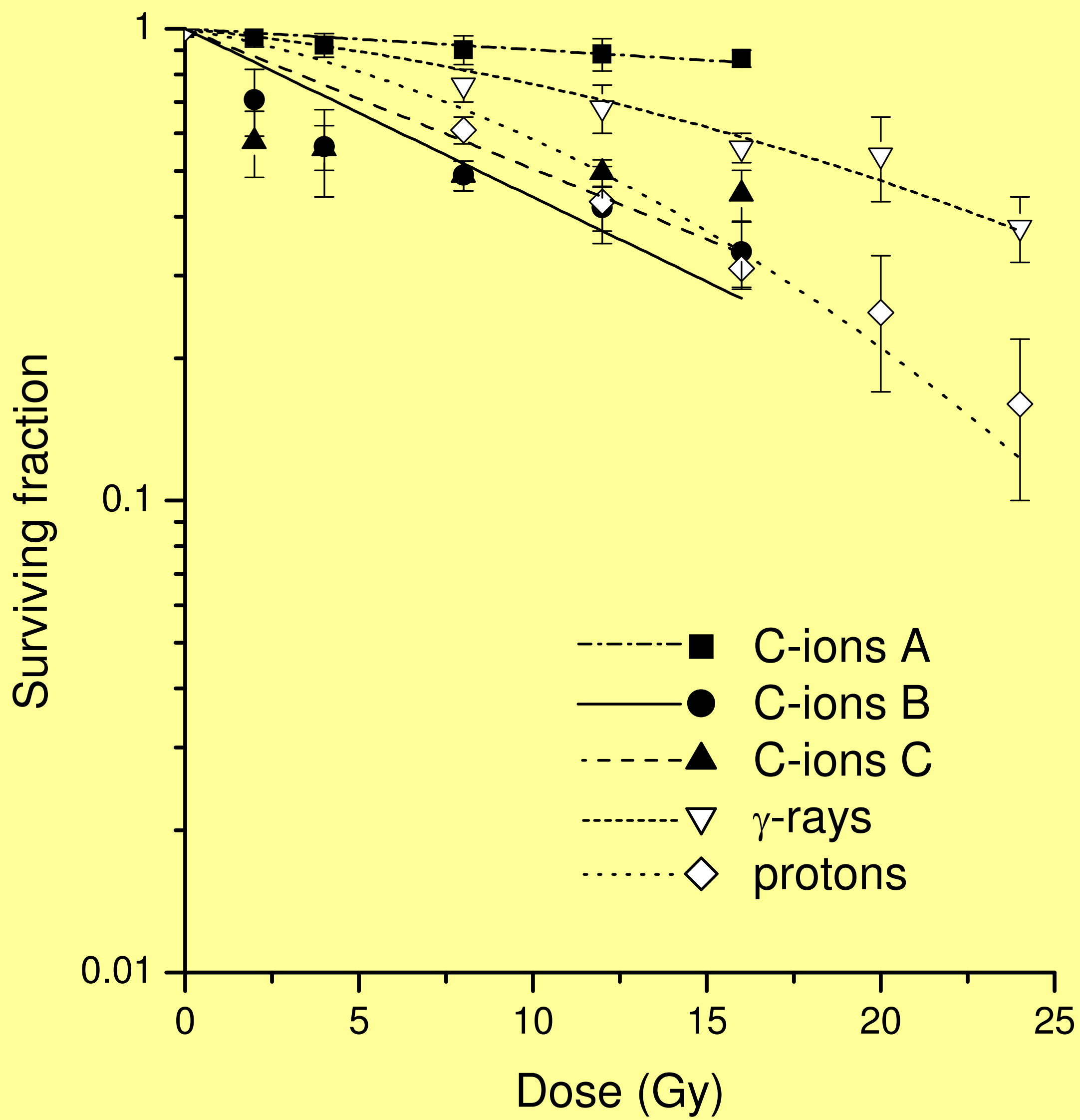
$$\mathbf{S = \exp(-\alpha D - \beta D^2),}$$

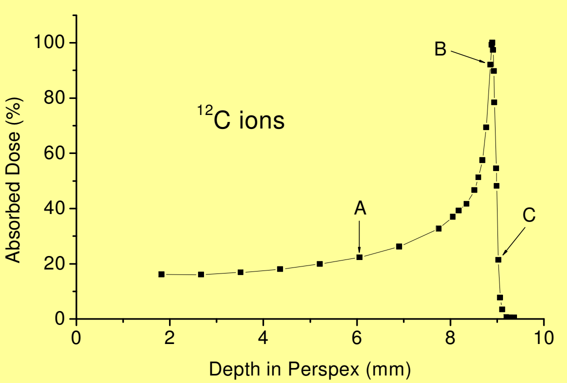
S - *surviving fraction having dose* **D,**

α *and* **β** - *free parameters.*



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





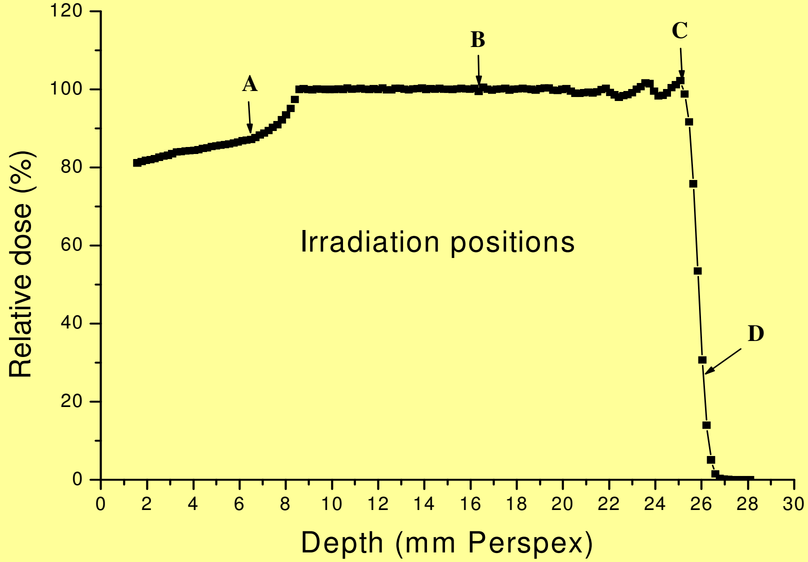
- Close to the end of range fluence falls down very fast to zero, while LET rises exponentially, producing the distal declining end of the Bragg curve.

- Consequently, the decreasing number of particles has leaser hits on irradiated cells. However, these hits by particles with increasing LET cause more irreparable and less reparable lesions.

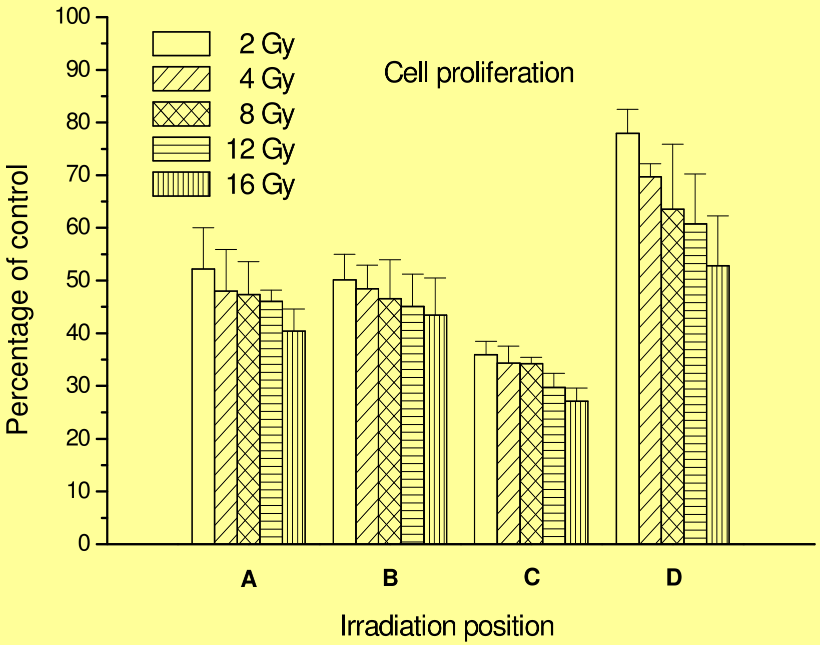
- Therefore, there is a relatively higher killing ability of a smaller number of particles when approaching the end of range. This explains why dose dependant survival curves for ^{12}C ions at irradiation positions B and C are close although there is an important difference in dose levels.

- Stronger killing ability at the Bragg peak of ^{12}C ions compared to protons is the consequence of their higher LET (22.50 keV/μm *vs.* 9.08 keV/μm, respectively), while particles energy of ^{12}C ions and protons are quite close (8.97 MeV and 8.59 MeV, respectively).

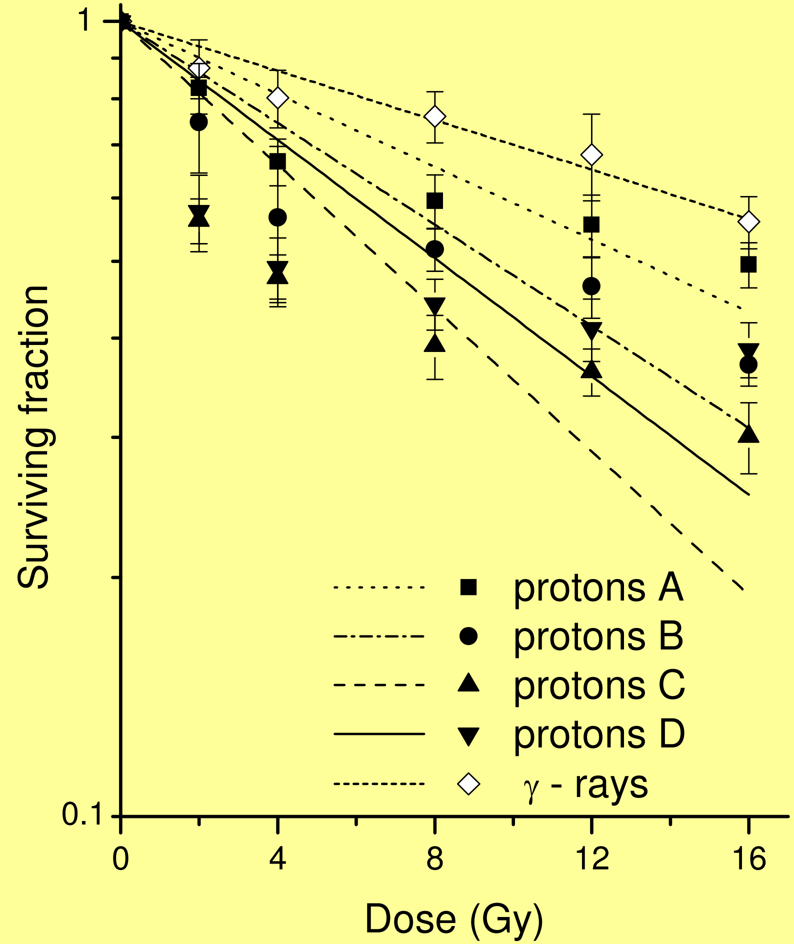
- Track structure of the energy deposition, including fragmentation and secondary particles, plays an important role and has to be taken into account when analyzing biological responses to different ion species. Therefore different biological responses are caused by the different track structure for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



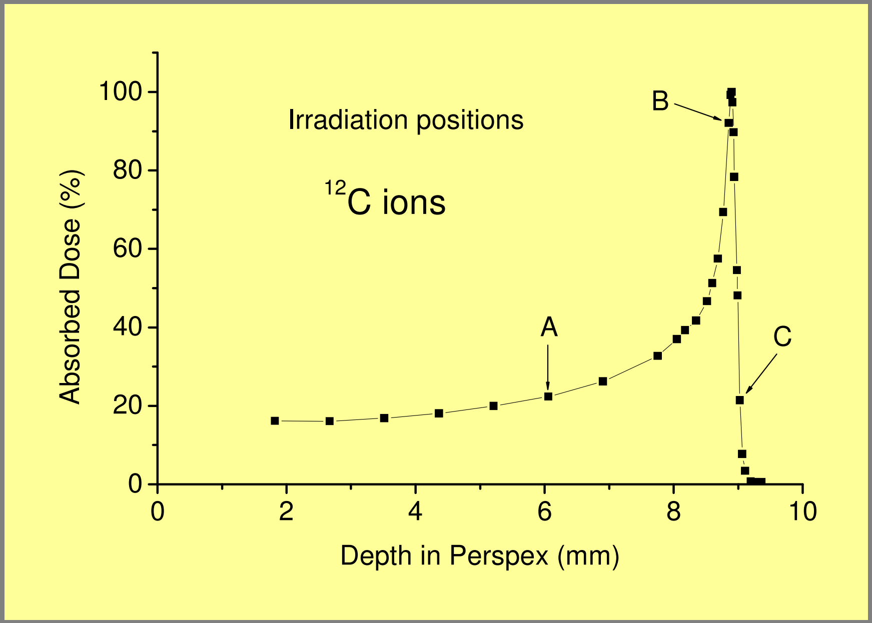
Relative dose (%)
Depth (mm Perspex)
Irradiation positions
A
B
C
D
0
20
40
60
80
100
120
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30

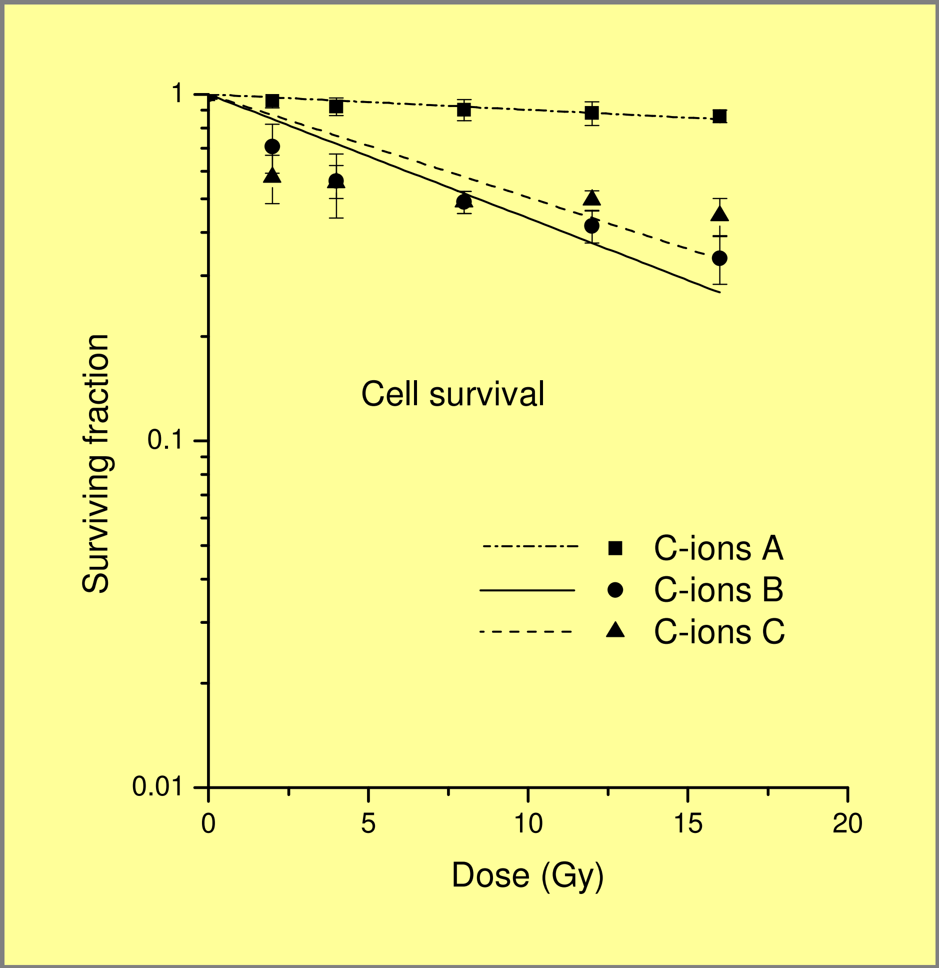


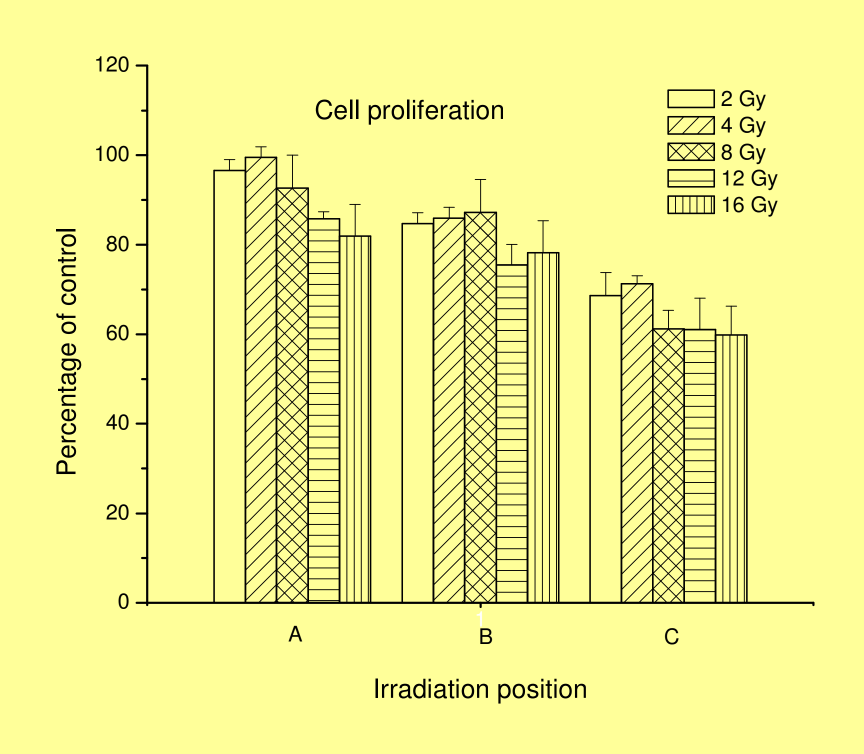
Percentage of control
Irradiation position
Cell proliferation
2 Gy
4 Gy
8 Gy
12 Gy
16 Gy
A
B
C
D
0
10
20
30
40
50
60
70
80
90
100



Surviving fraction
Dose (Gy)
protons A
protons B
protons C
protons D
γ - rays
1
0.1
0
4
8
12
16


Irradiation positions
^{12}C ions
Absorbed Dose (%)
Depth in Perspex (mm)
A
B
C
0
20
40
60
80
100
0
2
4
6
8
10


Cell survival
Surviving fraction
Dose (Gy)
C-ions A
C-ions B
C-ions C
1
0.1
0.01
0
5
10
15
20

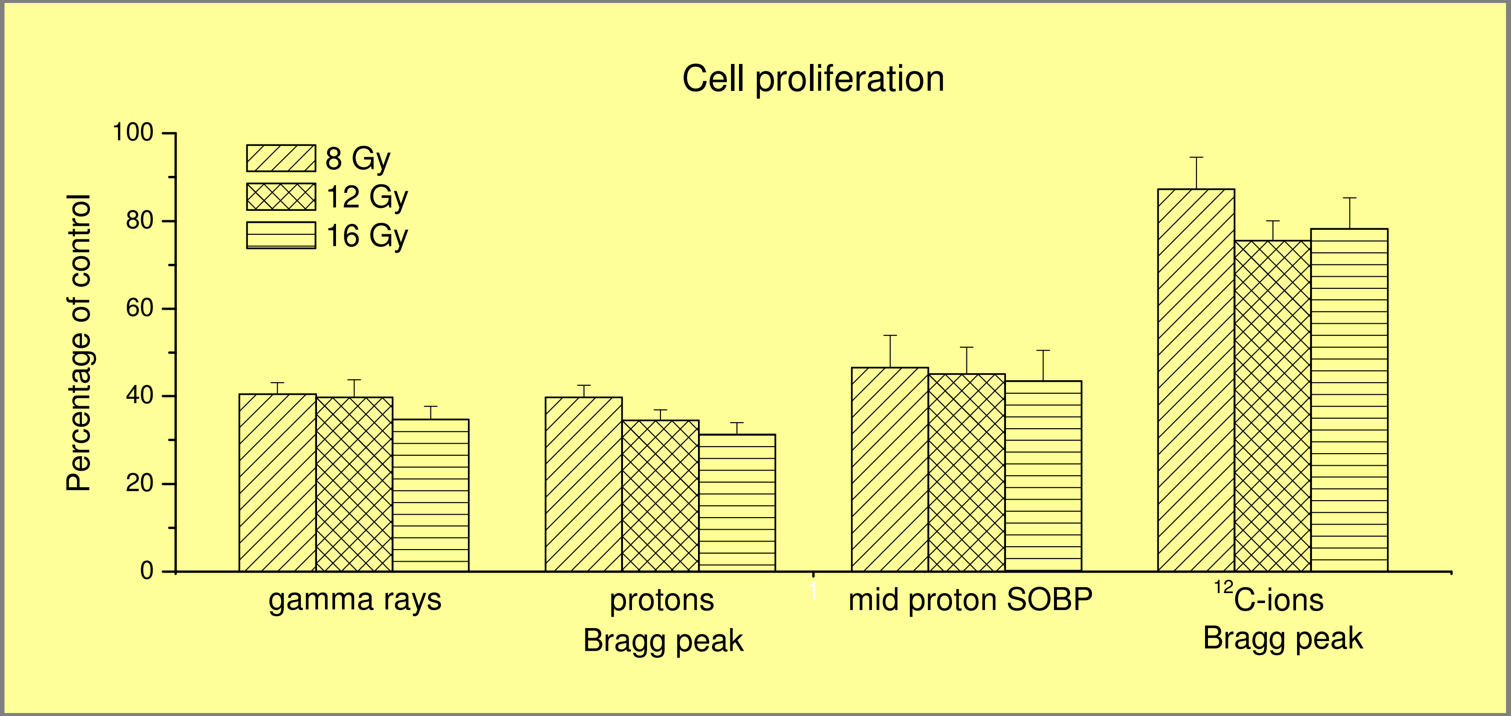

Cell proliferation
Percentage of control
Irradiation position
2 Gy
4 Gy
8 Gy
12 Gy
16 Gy
A
B
C
0
20
40
60
80
100
120

Radiobiological parameters of different types of radiations

Irrad.	Irrad. position	SF(2 Gy)	RBE(2 Gy, γ)
proton SOBP	A	0.825±0.061	1.39±0.06
	B	0.748±0.103	2.14±0.11
	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, γ)
^{12}C	A	0.979 ±0.001	-
	B	0.706±0.114	3.38±0.64
	C	0.576 ±0.092	3.29 ±0.58

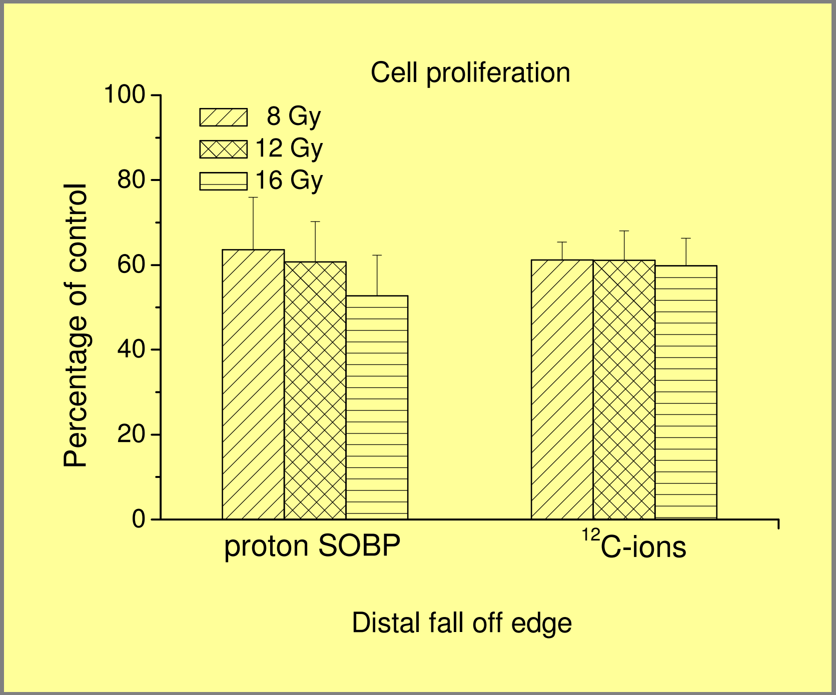
Irradiation	SF(2 Gy)	RBE(2 Gy, γ)	RBE(8 Gy, γ)	RBE(12 Gy, γ)	RBE(16 Gy, γ)
γ-rays	0.961±0.004	1	1	1	1
protons	0.931±0.006	1.69±0.17	1.61±0.09	1.64±0.14	1.63±0.13
mid SOBP	0.748±0.103	2.14±0.11	3.16±0.87	3.26±0.79	2.89±0.59
^{12}C-ions	0.706±0.114	3.38±0.64	3.30±0.39	2.80±0.42	2.47±0.34



- Proliferation capacity, monitored through ability of irradiated cells to incorporate BrdU, indicates that γ-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}C ions, due to their higher LET, reveal that more irreparable, but less reparable lesions, are induced. This leads to higher level of replication.

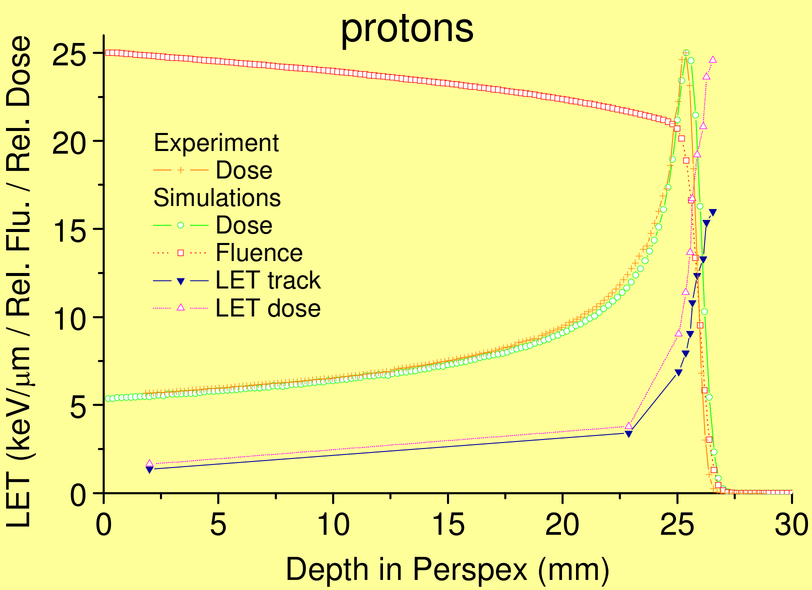
Another important concern is the effect of particles at the distal fall off part of the Bragg curve. This is particularly important for hadron therapy planning when a vital healthy tissue is located just behind the treated tumour.

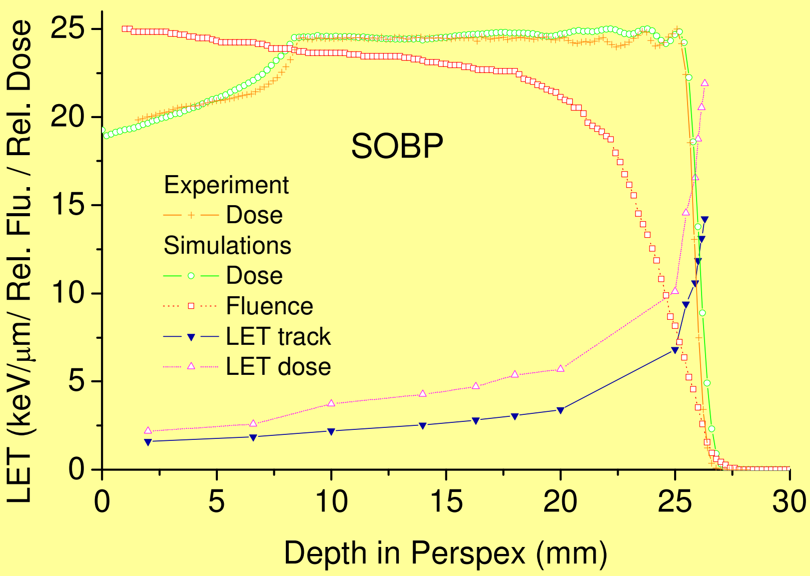


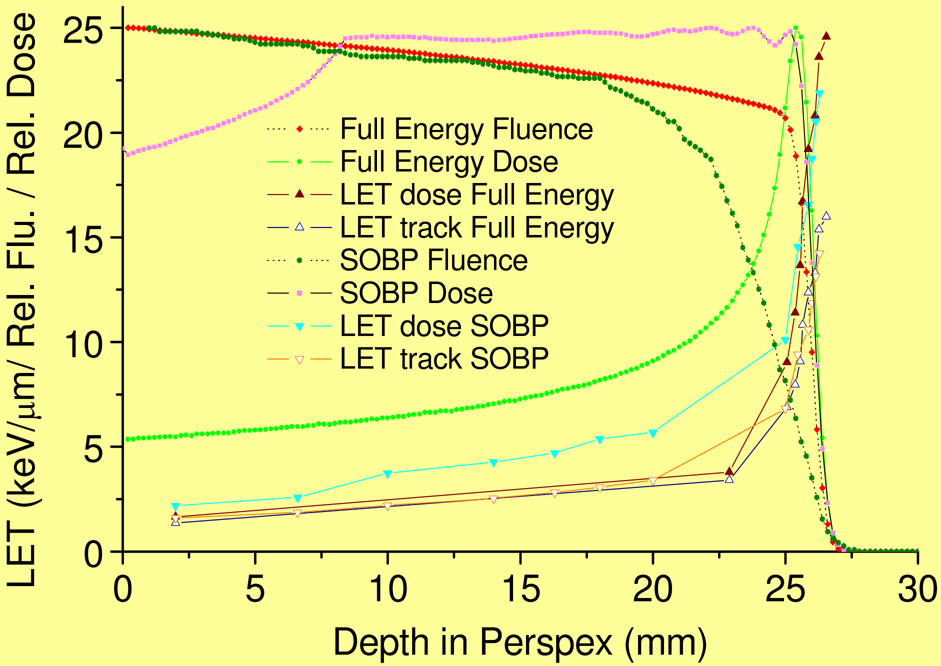
- Relatively high values of cell proliferation at the distal declining end of proton SOBP and ^{12}C ions Bragg curve are due to a smaller number of hits to irradiated cells and to a larger number of irreparable lesions induced by these hits. A greater number of cells can maintain active proliferation instead of being involved in complex repair processes.

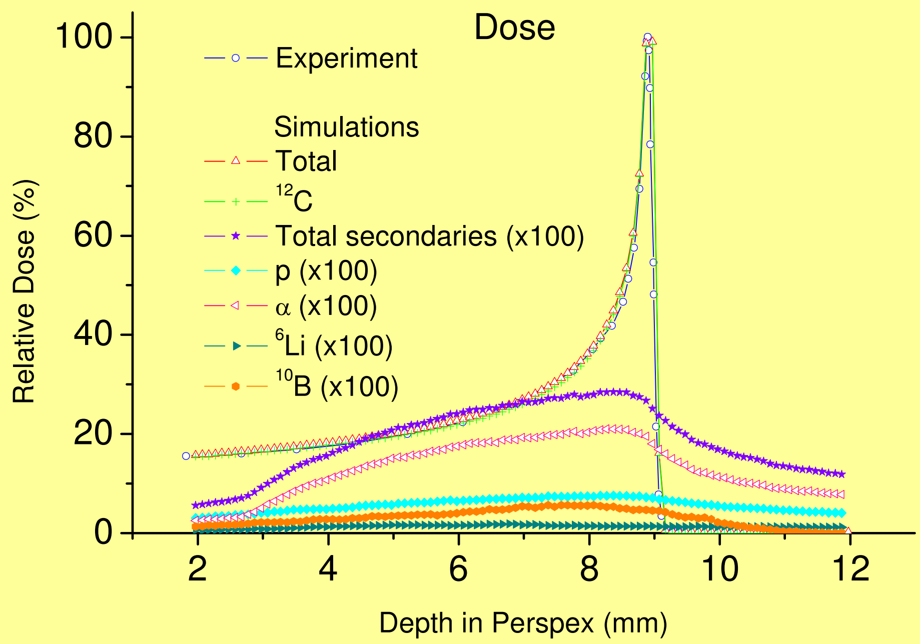
- Cell proliferation capacities at the irradiation positions at the distal declining end of the proton SOBP and of ^{12}C ions (with dose levels of 32.12±4.27 % and 21.42±6.43 %, respectively) are quite close, implying similar levels of irreparable and reparable lesions.

Numerical simulations with GEANT4 code

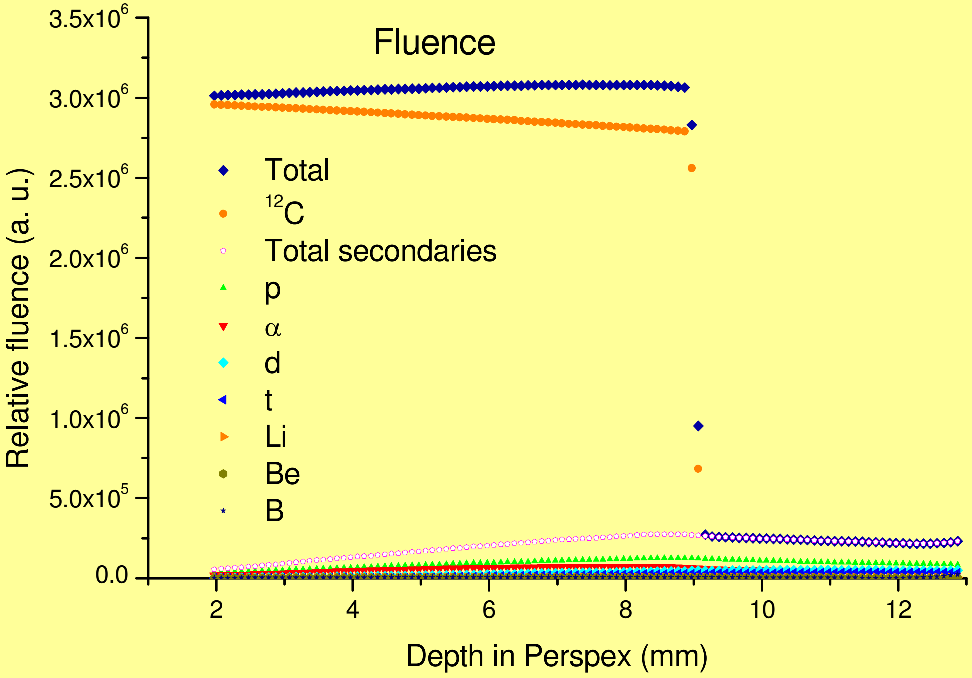

protons
LET (keV/μm / Rel. Flu. / Rel. Dose
Experiment
Dose
Simulations
Dose
Fluence
LET track
LET dose
Depth in Perspex (mm)


SOBP
LET (keV/μm/ Rel. Flu. / Rel. Dose
Experiment
Dose
Simulations
Dose
Fluence
LET track
LET dose
Depth in Perspex (mm)

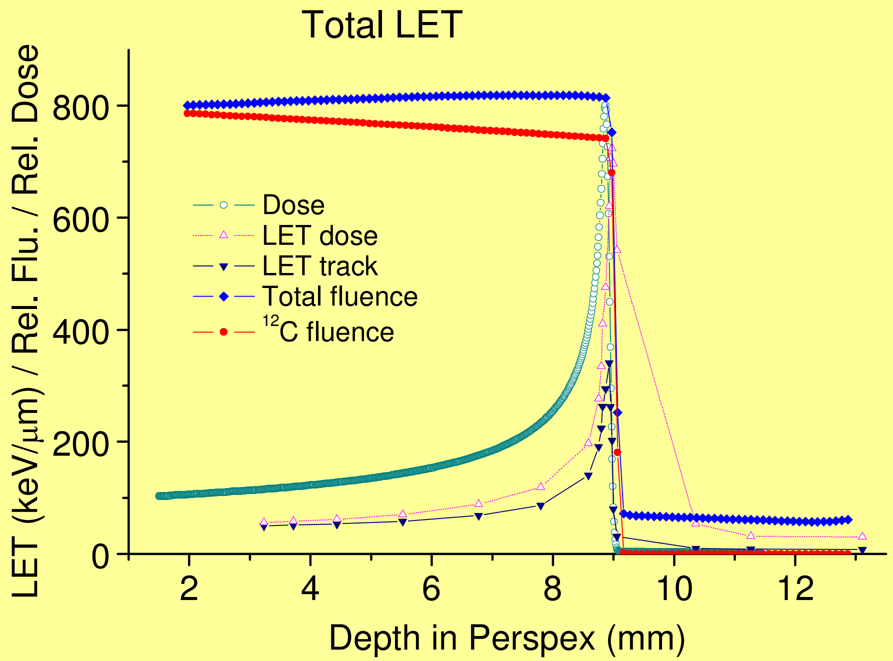

LET (keV/μm/ Rel. Flu. / Rel. Dose
Full Energy Fluence
Full Energy Dose
LET dose Full Energy
LET track Full Energy
SOBP Fluence
SOBP Dose
LET dose SOBP
LET track SOBP
Depth in Perspex (mm)

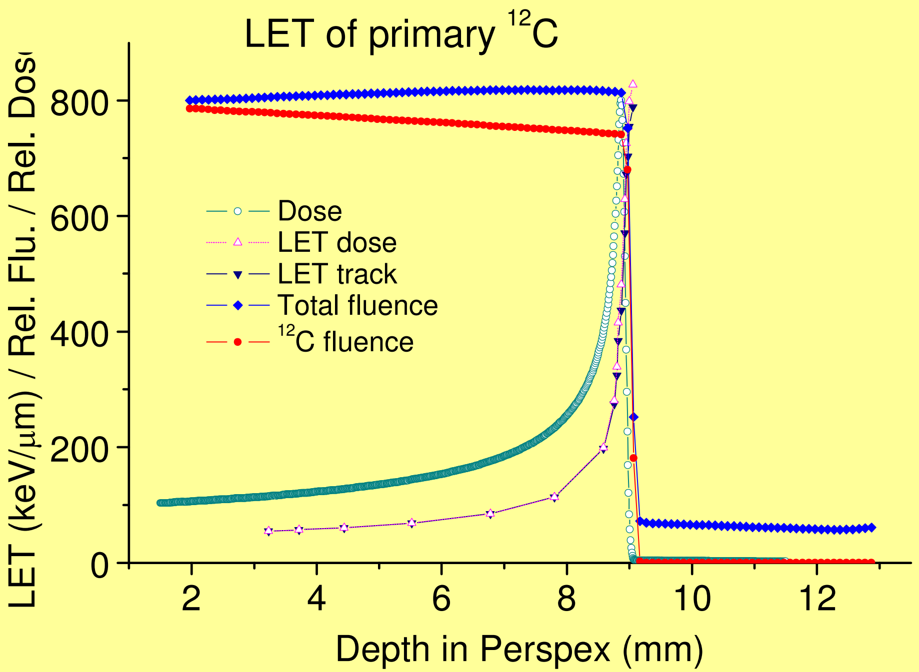


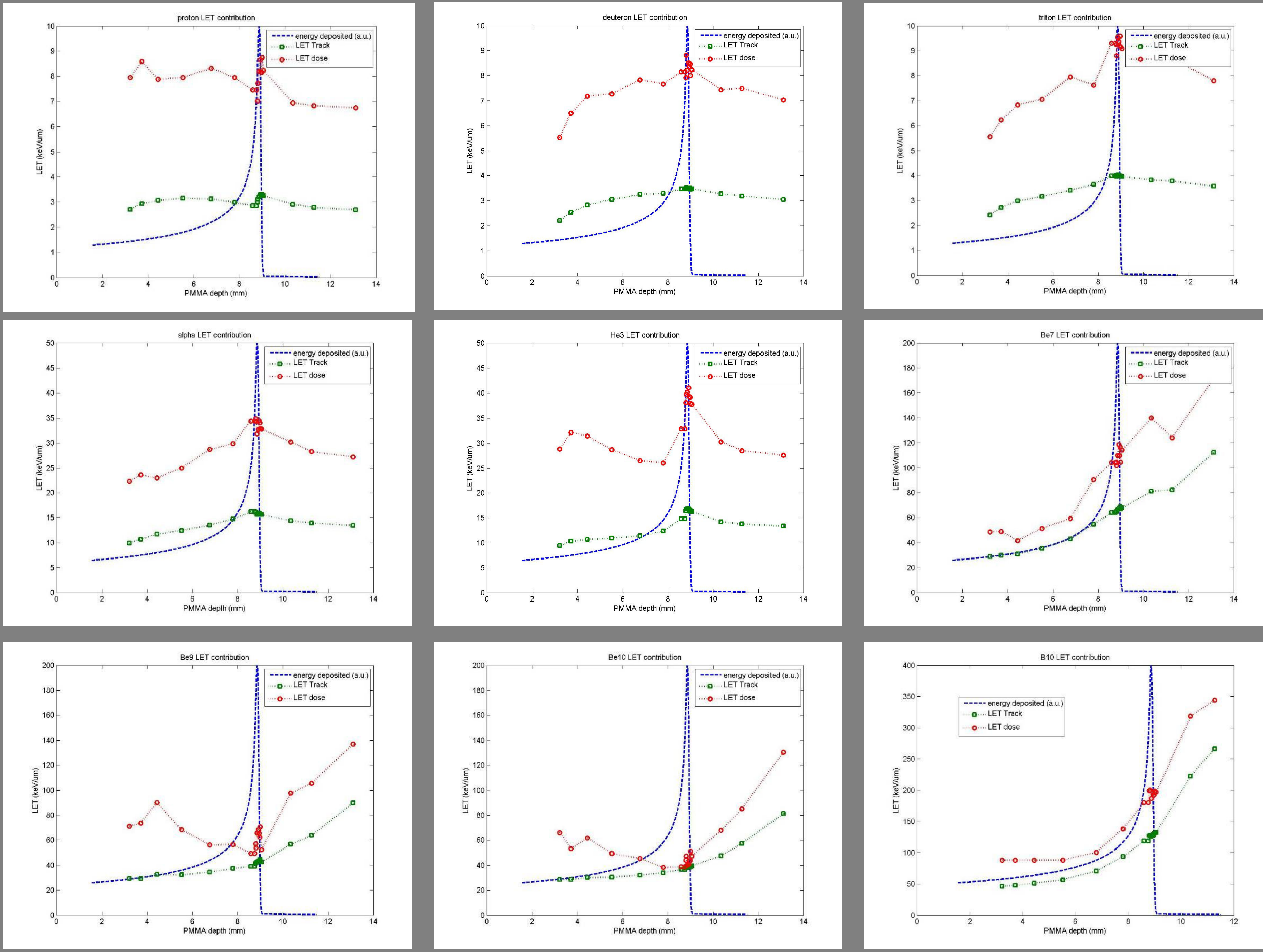
Dose
Relative Dose (%)
Depth in Perspex (mm)
Experiment
Simulations
Total
12C
Total secondaries (x100)
p (x100)
α (x100)
6Li (x100)
10B (x100)
0
20
40
60
80
100
2
4
6
8
10
12



Fluence
Relative fluence (a. u.)
Depth in Perspex (mm)
Total
12C
Total secondaries
p
α
d
t
Li
Be
B
0.0
5.0x10^5
1.0x10^6
1.5x10^6
2.0x10^6
2.5x10^6
3.0x10^6
3.5x10^6
2
4
6
8
10
12


Total LET
LET (keV/μm) / Rel. Flu. / Rel. Dose
Depth in Perspex (mm)
Dose
LET dose
LET track
Total fluence
12C fluence
0
200
400
600
800
2
4
6
8
10
12


LET of primary 12C
LET (keV/μm) / Rel. Flu. / Rel. Dos
Depth in Perspex (mm)
Dose
LET dose
LET track
Total fluence
12C fluence
0
200
400
600
800
2
4
6
8
10
12


proton LET contribution
deuteron LET contribution
triton LET contribution
alpha LET contribution
He3 LET contribution
Be7 LET contribution
Be9 LET contribution
Be10 LET contribution
B10 LET contribution
energy deposited (a.u.)
LET Track
LET dose
LET (keV/um)
PMMA depth (mm)

CONCLUSION

- After irradiations with γ-rays, protons and ^{12}C ions evaluated radiobiological parameters have shown high resistance of human HTB140 melanoma cells. Due to higher LET, ^{12}C ions have caused better effectiveness compared to protons.

- When receiving maximum dose, cells irradiated with γ-rays or protons, having lower LET, have shown lesser dose dependent proliferation than for ^{12}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}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}C ions could reach in producing biological response.