Sub-lethal cellular response of human normal cells along the Bragg curve for different ion beams: implications for human health and charged particle radiobiology

Lorenzo Manti

Department of Physics-Radiation Biophysics Laboratory

University of Naples Federico II & National Institute for Nuclear Physics, Naples Section, Italy

Why emphasis on charged particles

- Human exposure:
  - Environmental (indoor radon, Space)
  - Man-made (hadrontherapy, radioimmunotherapy)
- Risk estimates for patients and astronauts share radiobiological pillars, hence they suffer from common uncertainties
- Inaccuracies are twofold in nature: data paucity on long-term effects; inadequacy to model particle radiation action
  - Despite improved dose-shaping contours and tissue sparing, secondary cancers are of concern in hadrontherapy and cancer remains the main stochastic hazard for space exploration; non-cancer late effects also determine treatment outcome and may affect Space crews
  - Models based on dose and LET disregard ion-track structure, the key property that drives radiation quality effectiveness
- Both cancer and non-cancer effects originate from sub-lethally damaged cells
- How their response is related to the way particles deposit energy along (Bragg curve) and around (track structure) their path

Outline

- Particle radiobiology
- Sub-lethal cytogenetic damage
- Towards «biological» Bragg curves
- Conclusions

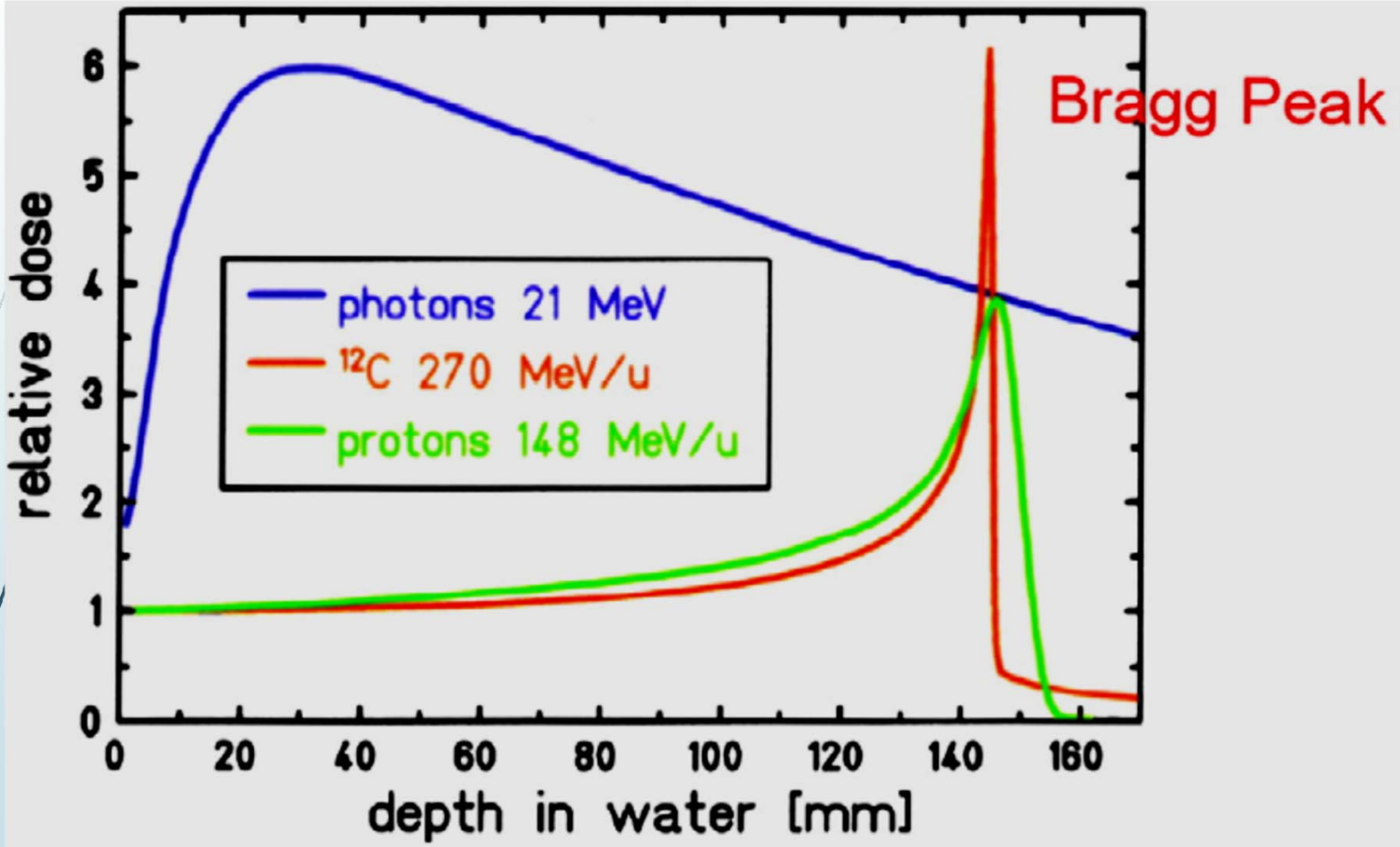
Particle radiobiology

- Ionising radiation is unique among all the mutagen and carcinogen agents
- The damage it induces (as a result of a complex cascade of biochemical processes at the molecular and cellular level) is a direct consequence of the mode with which energy is released along the radiation path
- The physical interaction pattern with the biological matter is the determinant of the consequences of radiation exposure

Particle radiobiology

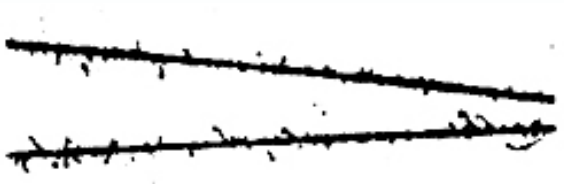
- Physical features
  - Bragg curve
  - Track structure
- Biological effectiveness
  - Complex DNA lesions
  - Effectively induced by charged particles, multiply damaged sites are believed to be the main reason for the higher RBE of particle-type radiation compared to photons

Particle radiobiology



Particle radiobiology

Highly inhomogeneous ionisation varieties and spurs mainly occur within and around the track, but also at the end of the single electrons put in motion by photon ionisation events.

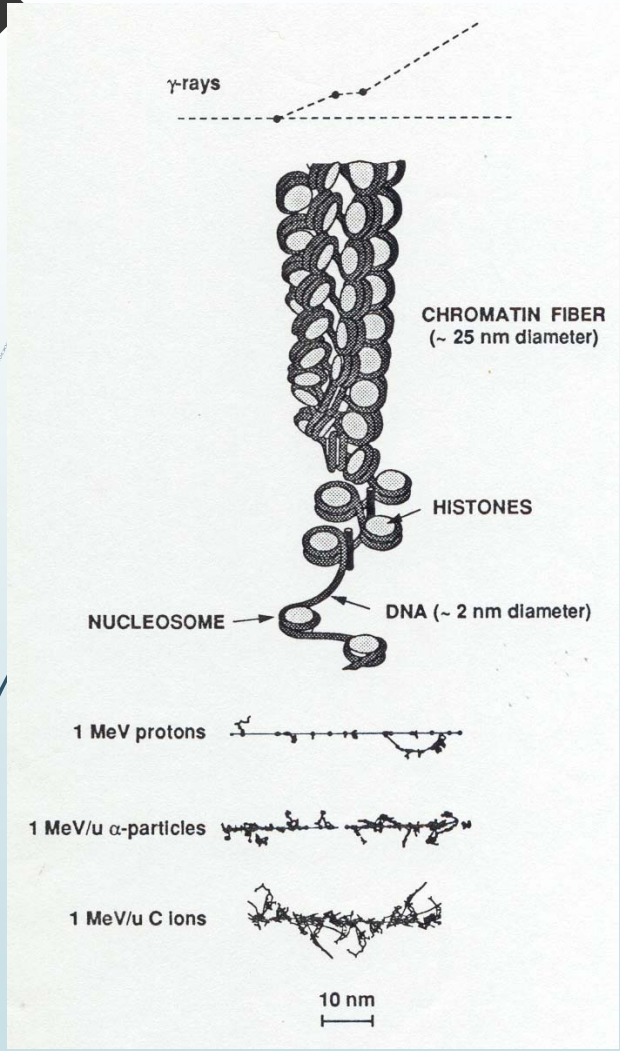


(a) Cloud chamber tracks of α-particles. The spurs on the outside of the tracks are the δ-rays, some of which have quite a considerable range.

b) Cloud chamber track of a 1 MeV electron, which shows that the ionizations occur in clusters.

(c) Cloud chamber tracks of a fast electron (*circa* 200kV) crossed by a dense track from a slow electron (*circa* 20kV); at this low energy the track is bent because of scattering.

Particle radiobiology

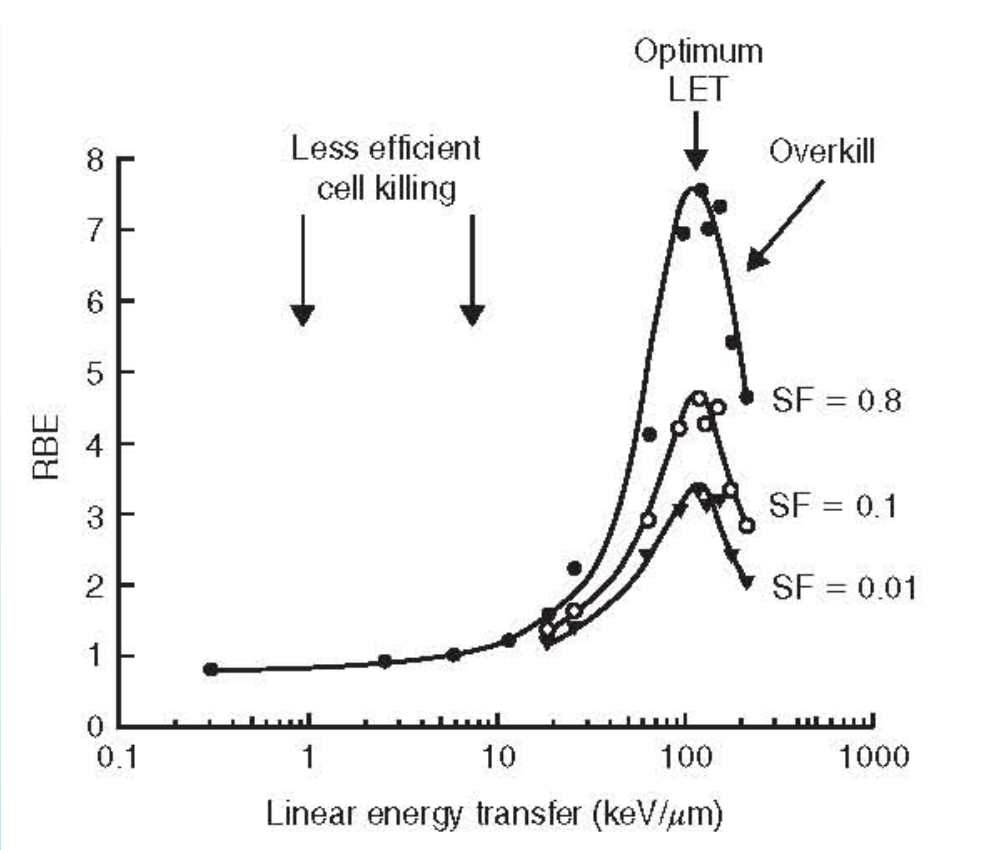


Closely spatio-temporally located lesions, encompassing breaks and base damages, are the hallmark of the stochastic and discontinuous energy deposition pattern, posing a strain to the repair machinery.

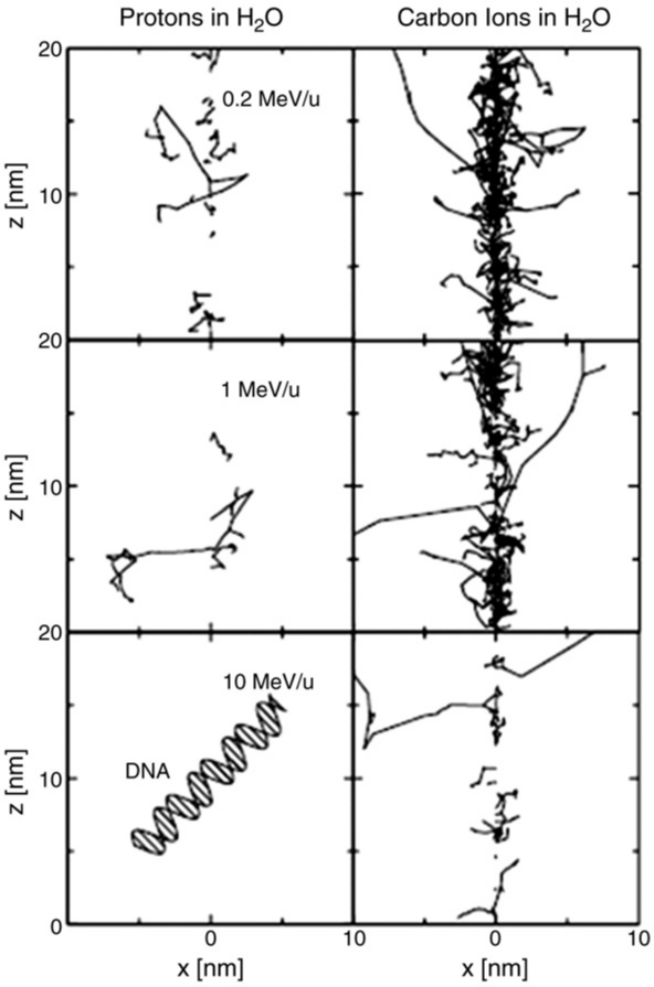
Particle radiobiology

LET alone not a satisfactory predictor of cellular response to particle radiation: Role of ion track structure

- R. Katz and F.A. Cucinotta, 1999
- C. Tsuruoka et al, 2005
- B.S. Sørensen, et al, 2011
- K. George et al., 2013



Particle radiobiology



Track diameter depends on energy and, for a given energy, on particle Z, hence ionisation patterns vary between same-LET ions

- Protons and carbon ions of similar LET values have different RBEs

Proton and carbon ion tracks are compared microscopically to an illustration of a DNA molecule before, in and behind the Bragg maximum, for the same energy

M. Kramer and G. Kraft, Calculations of heavy-ion track structure, Radiat. Environ. Biophys. 33, 91–109 (1994)

Particle radiobiology

- RBE depends on biological factors (cell type and cell-cycle position, oxygenation) but also on physical factors (particle type, dose deposition profile, microscopic energy distribution
- Unlike low-LET radiation, severity of charged particle-induced damage changes with the travelled depth
- RBE does vary along the ion track
  - One consequence is change in RBE across the treated volume in hadrontherapy

Outline

- Particle radiobiology
- Sub-lethal cytogenetic damage
- Towards «biological» Bragg curves
- Conclusions

Sub-lethal cytogenetic damage

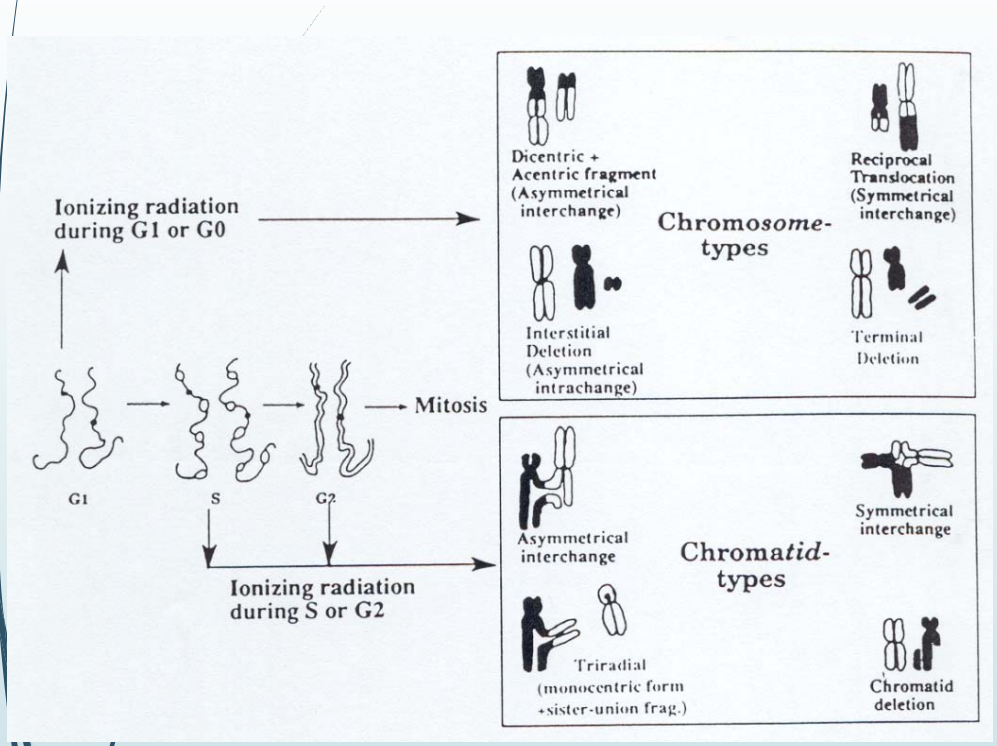
- Inaccuracy in RBE for long-term effects, its dependence upon ion type and position along the Bragg curve may limit hadrontherapy large-scale adoption and Space exploration
- Some of the identified health issues (yet not so for underlying mechanisms...)
  - Cancer (angiogenesis modulation)
  - Cardiovascular pathologies (pro-inflammatory responses)
  - Increased risk of thrombosis (von Willebrandt factor release)
  - Tissue and organ function (cytokine-driven degenerative processes)
  - Ageing and CNS disorders (on-going oxidative stress, decline in stem cell capability)

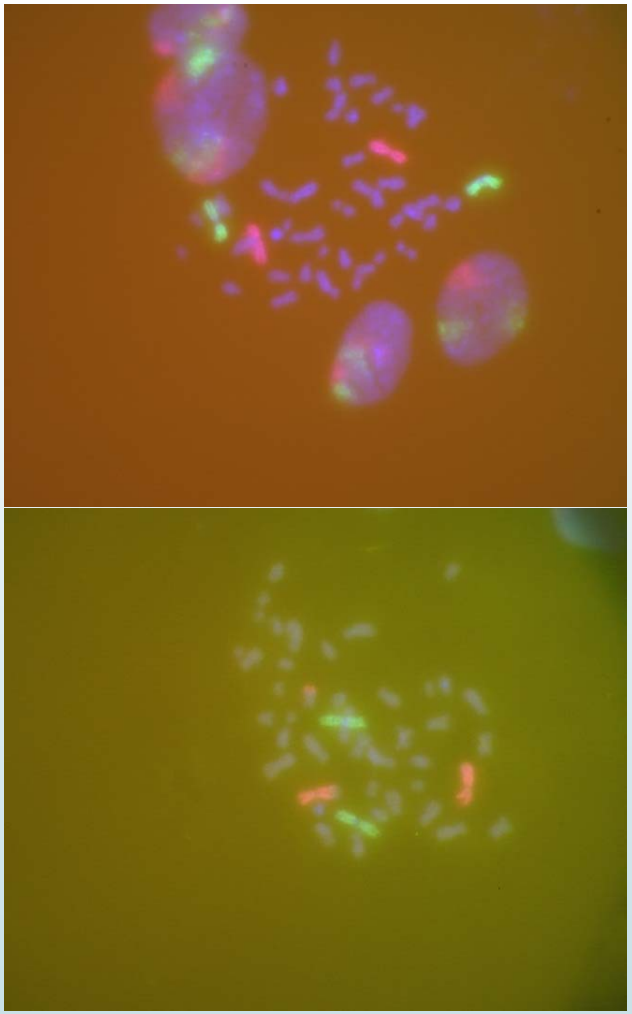
Andratschke et al., 2011; *D. Newhauser and M.Durante, 2011; A. Ottolenghi and K.R. Trott, 2011*

Sub-lethal cytogenetic damage

- Until recently, focus was primarily on cell killing/carcinogenesis, mainly from the Bragg peak region (mostly SOBP in therapeutic settings) or at the beam entrance
- Need for data on sub-lethal damage, such as cellular premature senescence and chromosome aberrations (CAs), along the Bragg curve and for different ion types
  - ✓ Accumulation of prematurely senescing cells may disrupt tissue homeostatic balance, impair organ function, exacerbate late tissue reactions and influence tumour niche cell proliferation kinetics
  - ✓ CAs increase neoplastic transformation risk instability, fuel genomic leading to cancer predisposition but also to multi-organ failure, and inflammatory responses, in addition to providing mechanistic information on track structure dependence

Sub-lethal cytogenetic damage





- ✓ Arise from mis- or unrepaired DNA damage
- ✓ Signature of ion exposure
- ✓ Well-established biomarker of cancer risk

Sub-lethal cytogenetic damage

- Chromosome aberrations
  - S. Ritter and M. Durante, Heavy-ion induced chromosomal aberrations: A review, Mutat. Res., 701, 38–46 (2010)
  - K. George et al. Biological effectiveness of accelerated particles for the induction of chromosome damage: track structure effects, Radiat. Res.,180, 25–33 (2013)
- Premature cellular senescence

Sub-lethal cytogenetic damage

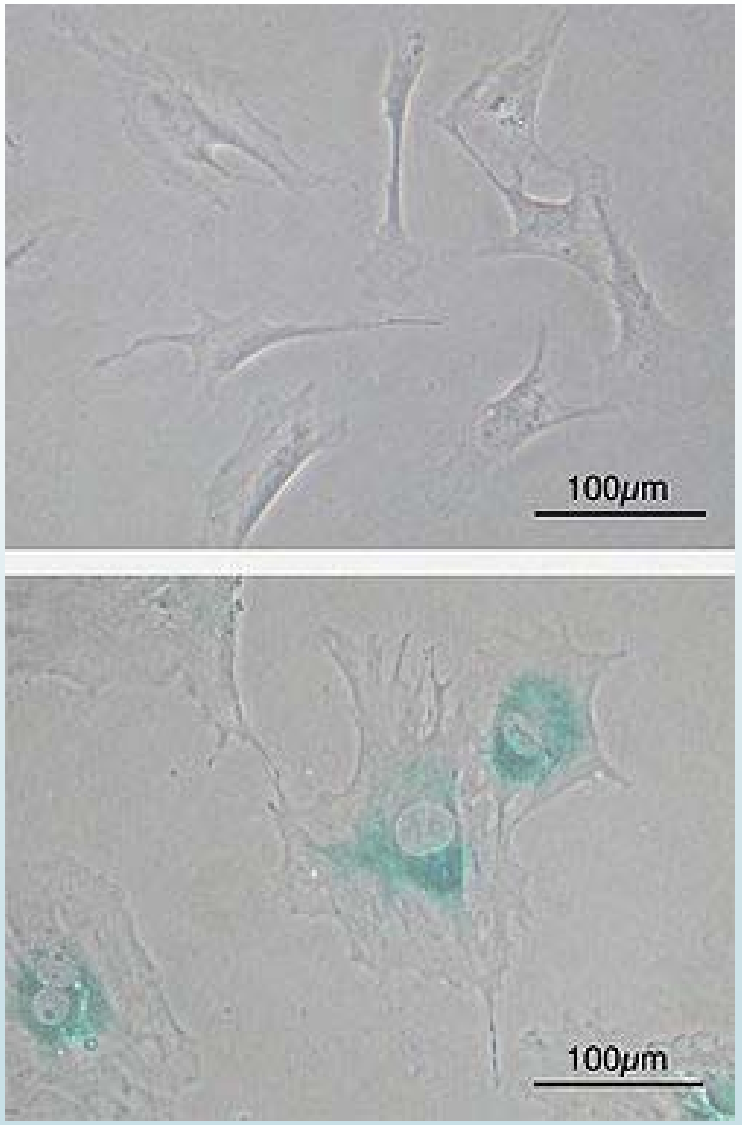
- Replicative senescence (RS) was first reported by Hayflick e Moorhead (*The serial cultivation of human diploid cell strains.* Exp. Cell Res., 1961)
  - *Cultured cells do not proliferate indefinitely but enter a metabolically active state of irreversible growth arrest (* 60÷80 *population doublings*)
- Overcoming the dogma by which all cells given optimal growth conditions can endlessly proliferate led to postulate that mechanisms causing exhaustion of proliferative potential may be expression of intracellular processes (pre-determined lifespan)
- These observations were instrumental to identify RS as the natural fate for the cell, a fate only tumour cells can elude, and to see that cellular senescence acts as a physiological barrier to tumorigenesis
  - Xue et al., Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas, Nature Letters,445, 656-660 (2007)
  - R.J Sabin and R. M. Anderson, Cellular Senescence - its role in cancer and the response to ionizing radiation, Genome Integrity, 2, 2-9 (2011)

Sub-lethal cytogenetic damage

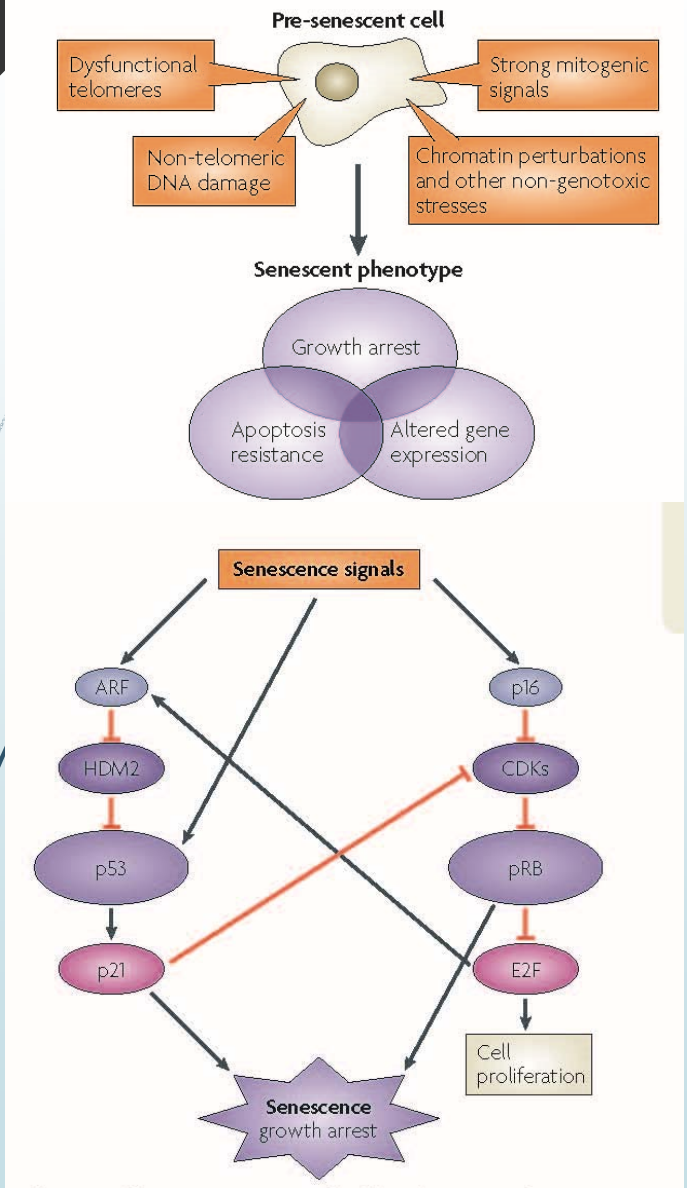
Morphological, biochemical and cellular hallmarks of RS

- Cell flattening
- Specificity for β-galactosidase
- *Over-expression* of p15, p16Ink4a (p16), p19 e p21Cip1/Waf1 (p21)
- Rb hypophosphorylation
- Resilience to apoptosis
- DNA *damage foci* persistence
- SAHF (*senescence-associated heterochromatic foci*)
- SAPS (*senescence-associated secretory phenotype*)

Mechanistically associated with telomere attrition



Sub-lethal cytogenetic damage



- A variety of sublethal cyto- and genotoxic insults may lead cells to senesce prematurely, a phenomenon known as *Stress-Induced Premature Senescence* **(SIPS)**
  - Oxidative stress (H_2O_2, hyperoxia), UV, ionising radiation, etc.
- Reminiscent of RS, not necessarily permanent
  - Telomere shortening controversial
- Shared pathways (p53 & p16)
- DNA damage response (DDR) appears to be a common effector of RS and SIPS

J. Campisi and F. d'Adda di Fagagna, Nature Reviews, 2007

Sub-lethal cytogenetic damage

- Age-related increase in ROS and decline in DNA repair capacity as major components of DNA damage accumulation and organismal ageing
  - Less clear as to how the antioxidant defence systems influence increased accumulation of DNA damage during ageing
- At cellular level, damage results in senescence or apoptosis, leading in turn to compromised tissue homeostasis through stem cells depletion and and/or disrupted tissue architecture
- Organ function decline manifesting phenotypically as organismal ageing

7420 Nucleic Acids Research, 2007, Vol. 35, No. 22

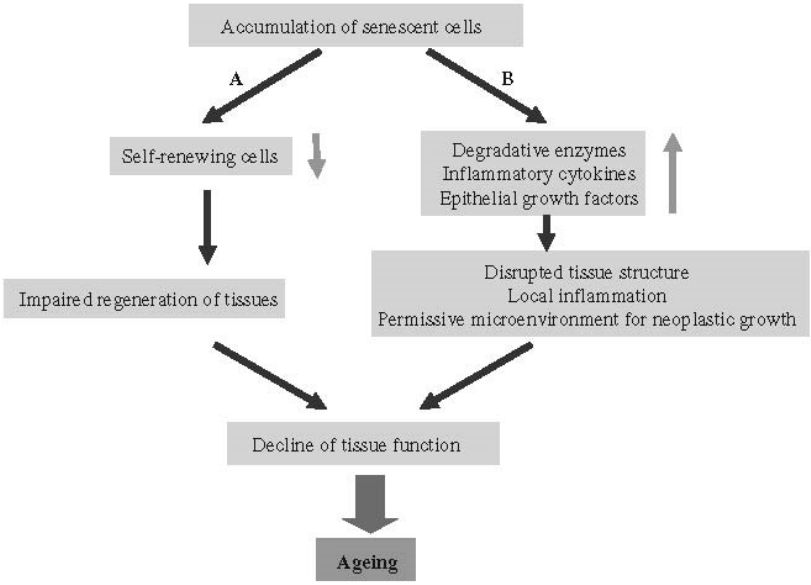


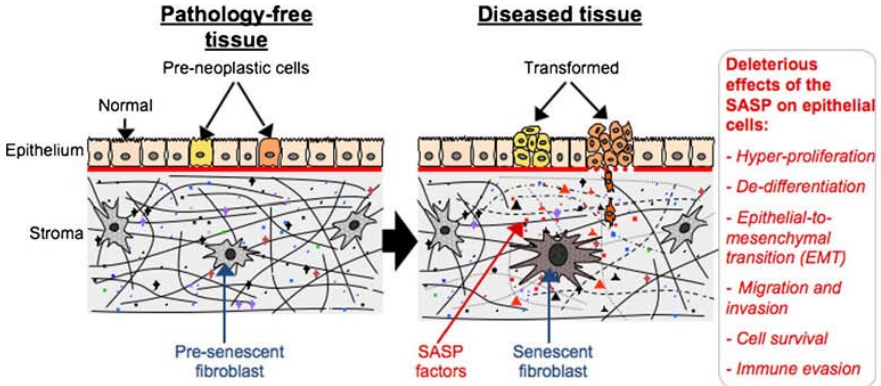
Figure 2. Two possible pathways through which cellular senescence may contribute to the ageing process. **(A)** Cellular senescence may reduce self-renewing cells, thus causing impaired regeneration of tissues. **(B)** Cellular senescence may cause disrupted tissue structure, local inflammation and permissive microenvironment for neoplastic growth through secretion of degradative enzymes, inflammatory cytokines and epithelial growth factors. Both pathways can cause compromised tissue homeostasis and function which ultimately lead to ageing.

Chen et al.

Sub-lethal cytogenetic damage

- Senescence–Associated Secretory Phenotype (SAPS): secretion of factors released by cells undergoing SIPS have been associated with either the inhibition or the promotion of cellular proliferation in surrounding tumour cells
  - K. K. C. Tsai et al., *Low-dose radiation-induced senescent stromal fibroblasts render nearby breast cancer cells radioresistant*. Radiat. Res., 172, 306-313 (2009)
  - A. R. Davalos et al.,, *Senescent cells as a source of inflammatory factors for tumor progression*. Cancer Metastasis Rev., 29, 273-283 (2010)

Fig. 2 Pro-tumorigenic paracrine effects of senescent cells. Senescent stromal fibroblasts can promote various facets of cancer progression (*right panel*). Pre-neopastic or transformed epithelial cells are shown in *dark color*; senescent cells cells are represented in *dark gray*. Pre-senescent and senesent fibroblasts secrete SASP factors that can promote cancer progression and aggressiveness



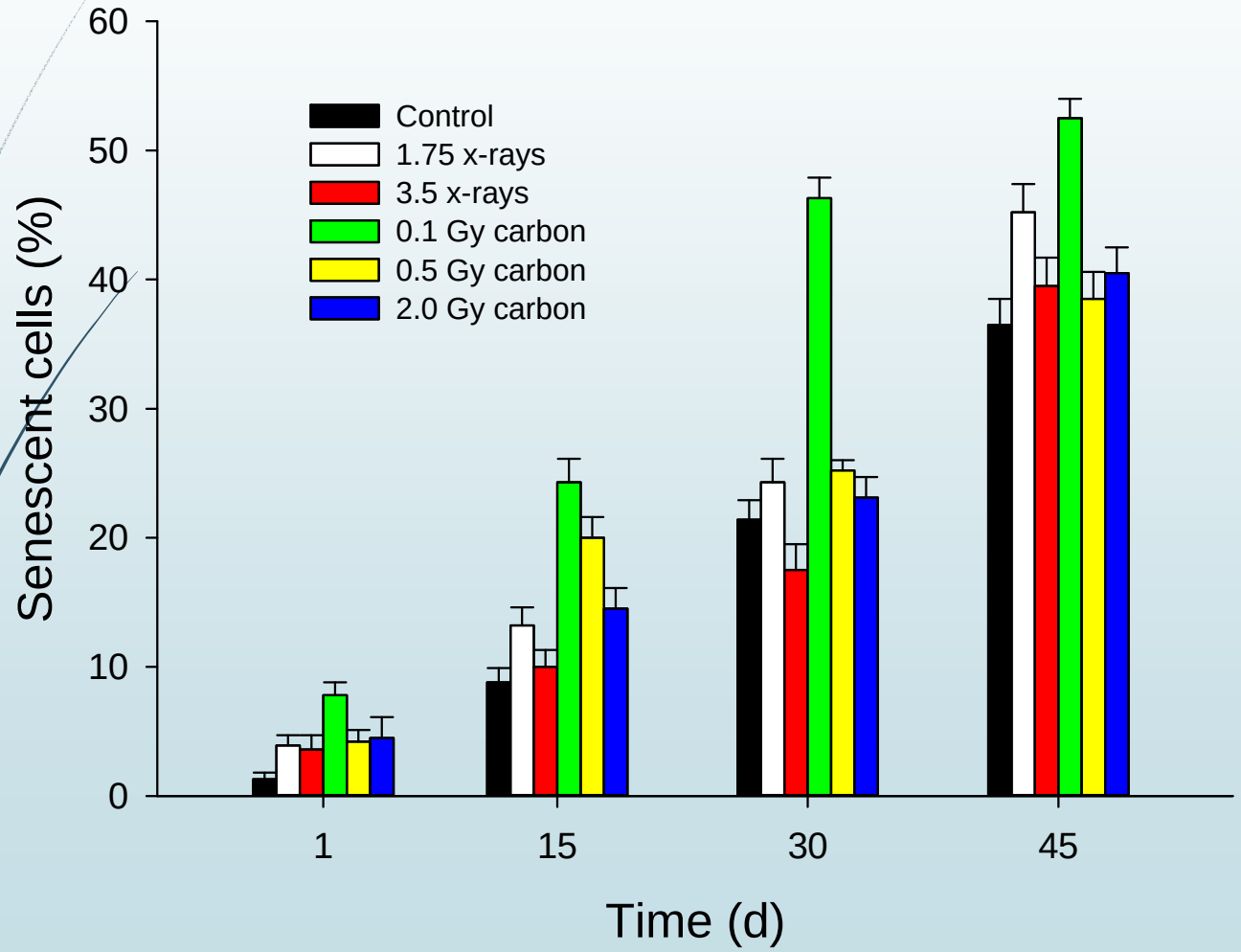
Springer

Sub-lethal cytogenetic damage

- Evidence for a greater RBE of particle radiation for SIPS
  - Suzuki et al. (*Radiat. Res.,164, 505-508, 2005*) reported a reduction in the life-span of human fibroblasts exposed to chronic low-dose exposure to a mixed field of heavy ions
  - Data on telomere loss and dysfunction (see Q. Zhang *et al., Radiat. Res., 164, 497-504,* 2005) also suggest that heavy ions can elicit premature senescence more efficiently than sparsely ionising radiation
  - Fournier et al. (Radiother. Oncol., 83, 277-282, 2007) observed premature differentiation, senescence and genomic instability in long-term cultures of human fibroblasts following ^{12}C ions

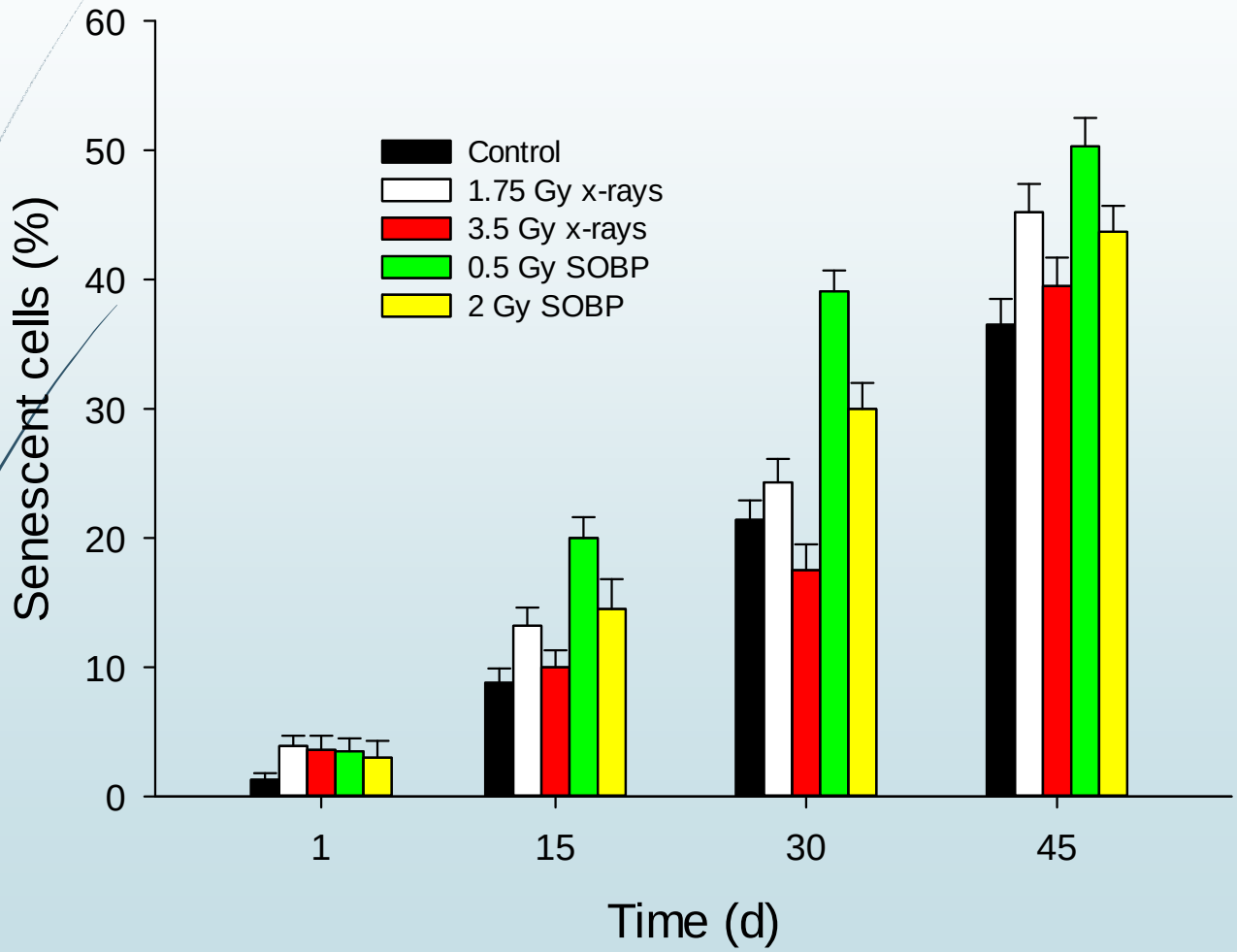
Sub-lethal cytogenetic damage

GSI carbon beam-plateau irradiation (13 keV/micron)



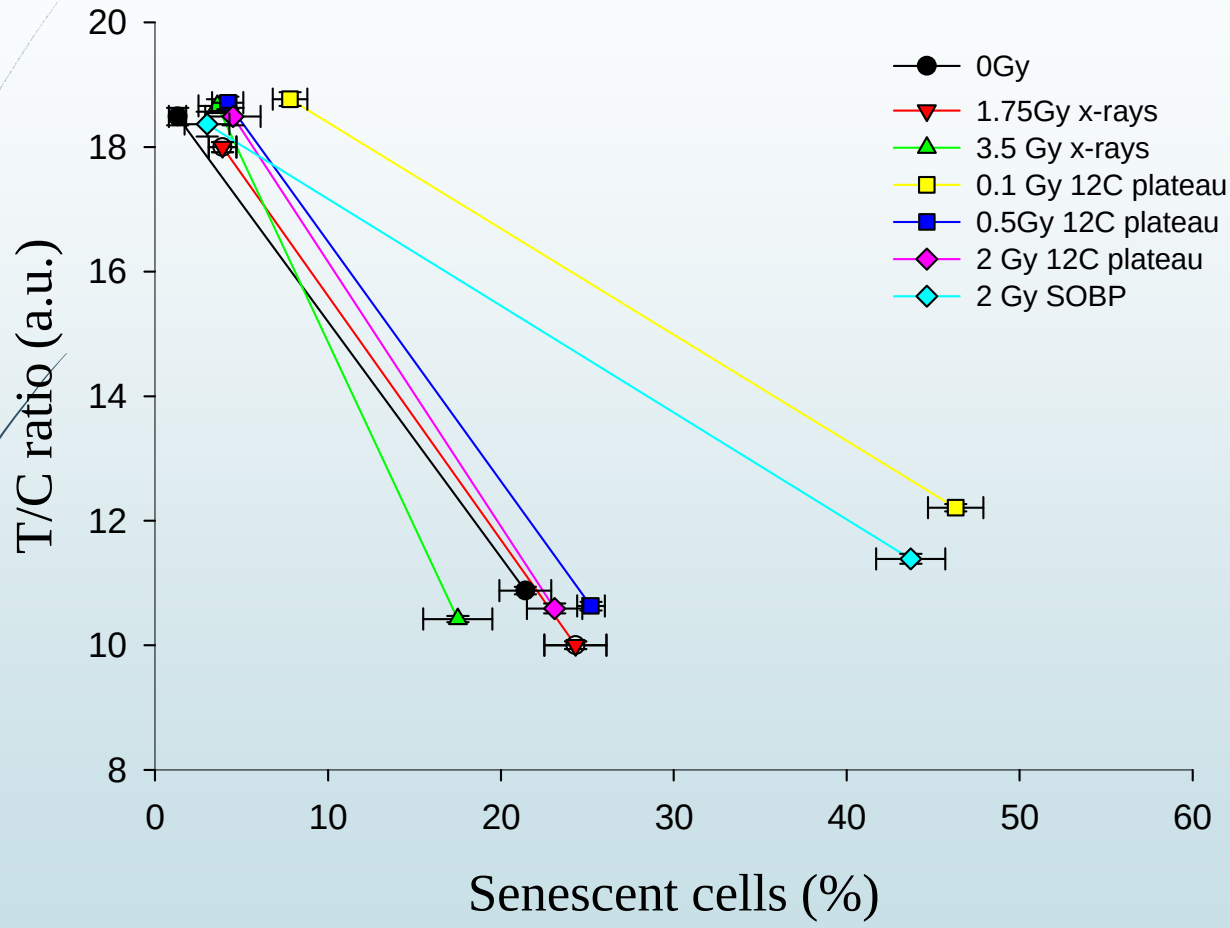
Sub-lethal cytogenetic damage

GSI carbon irradiation-mid SOBP (100 keV/micron)



Sub-lethal cytogenetic damage

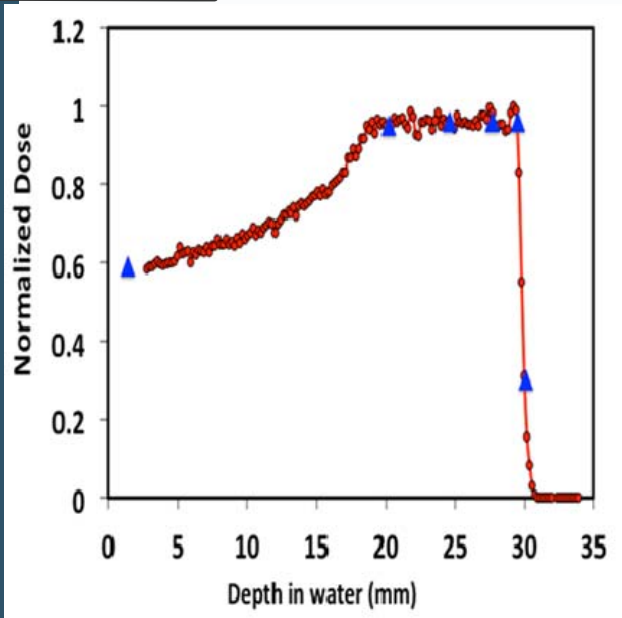
Relative telomere length vs cellular senescence at days 1 and 30

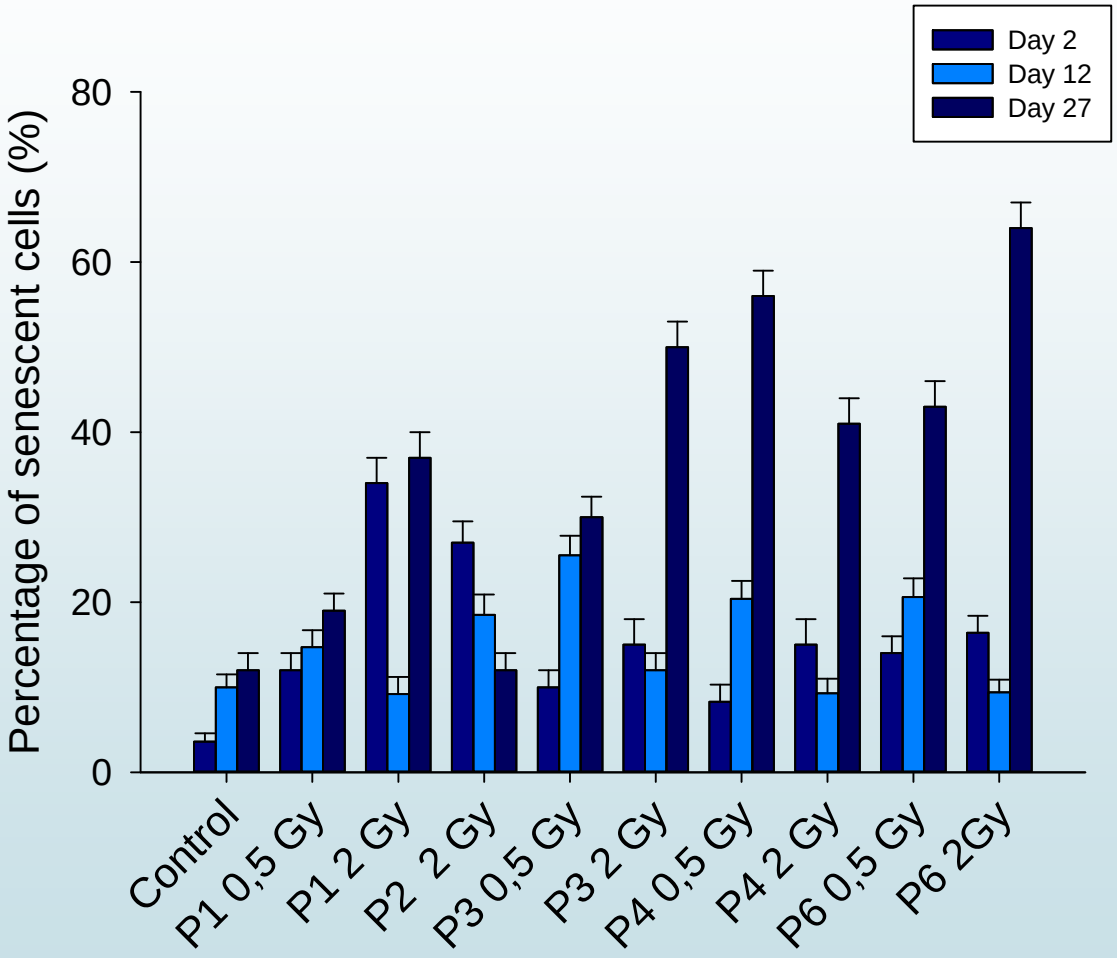


Outline

- Particle radiobiology
- Sub-lethal cytogenetic damage
- Towards «biological» Bragg curves
- Conclusions

Towards «biological» Bragg curves



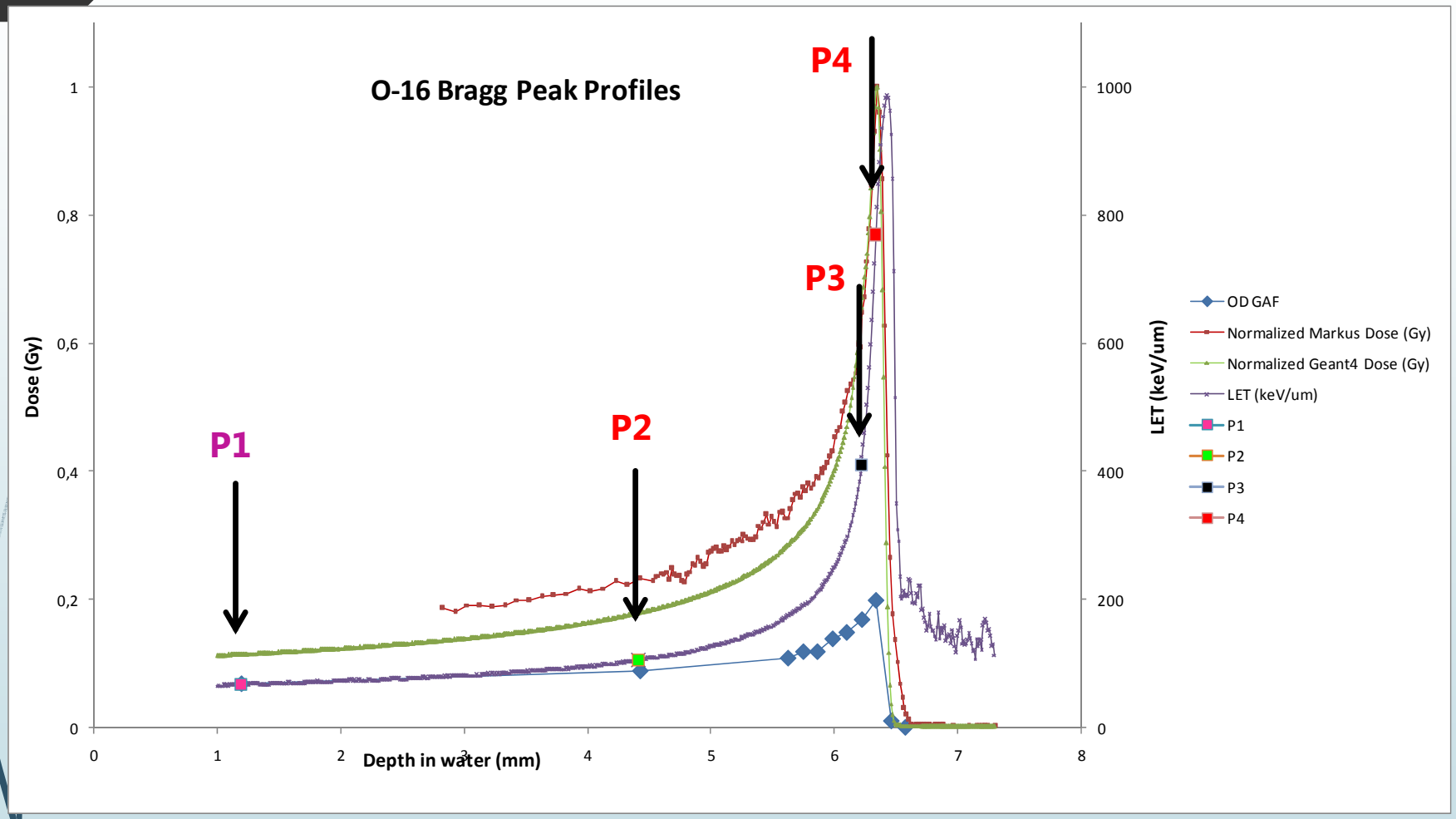


Therapeutic proton beam at LNS-INFN Catania

Towards «biological» Bragg curves

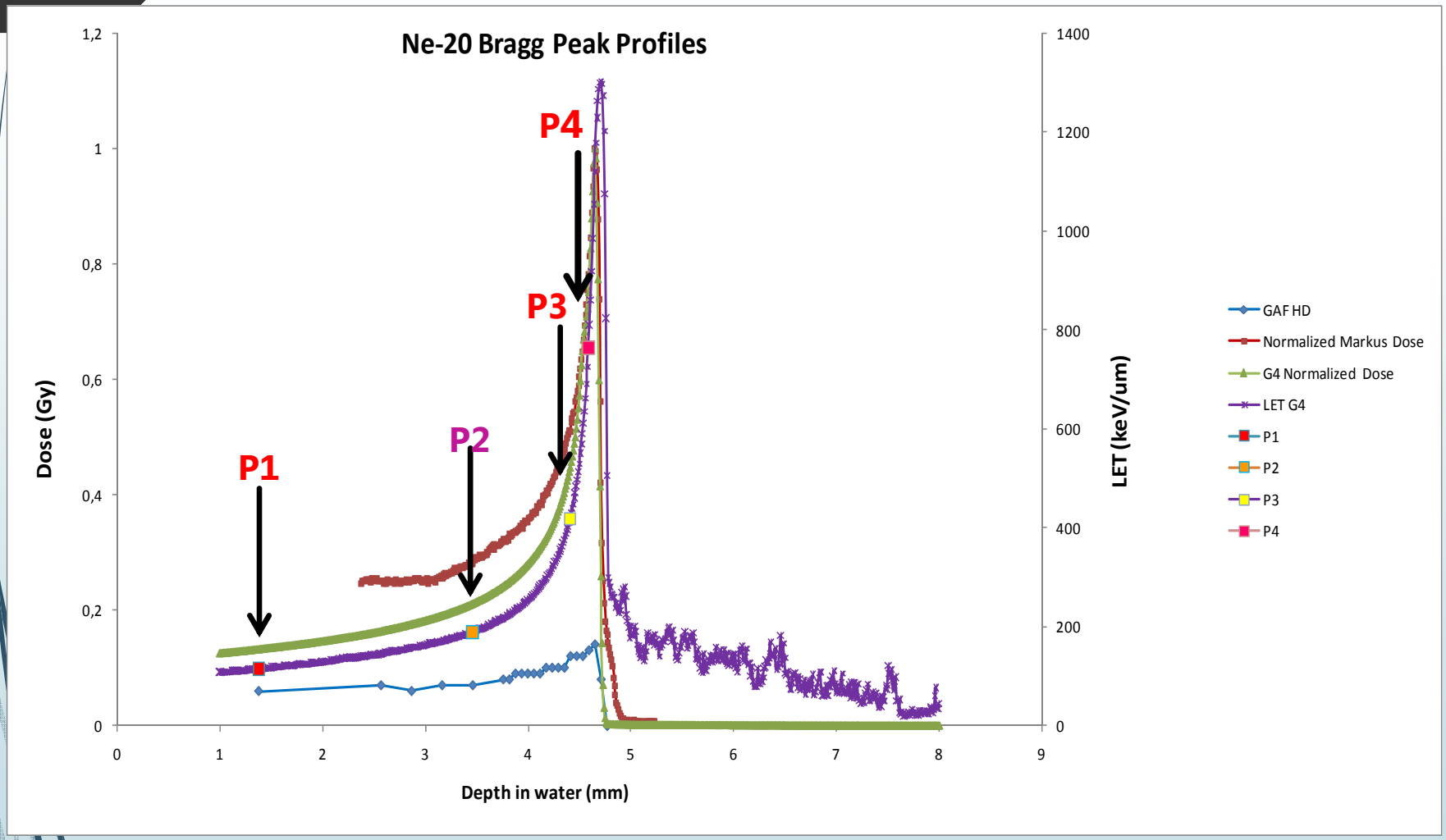
- Irradiations with 60 MeV/u ^{16}O and 58 MeV/u ^{20}Ne beams at LNS-INFN cyclotron (Catania, Italy)
- Dosimetry and Monte-Carlo simulations
  - Dose distributions (Markus chamber) and relative LET values (Geant4 simulations)
- Four positions along the pristine Bragg curves
- Sample positioning verified by optically reading of Gafchromic films and comparing the qualitative dose profile with the quantitative measurements from the Markus chamber.
  - Sample positioning was achieved with resolution less than 50 μm greatly reducing dose uncertainties

Towards «biological» Bragg curves



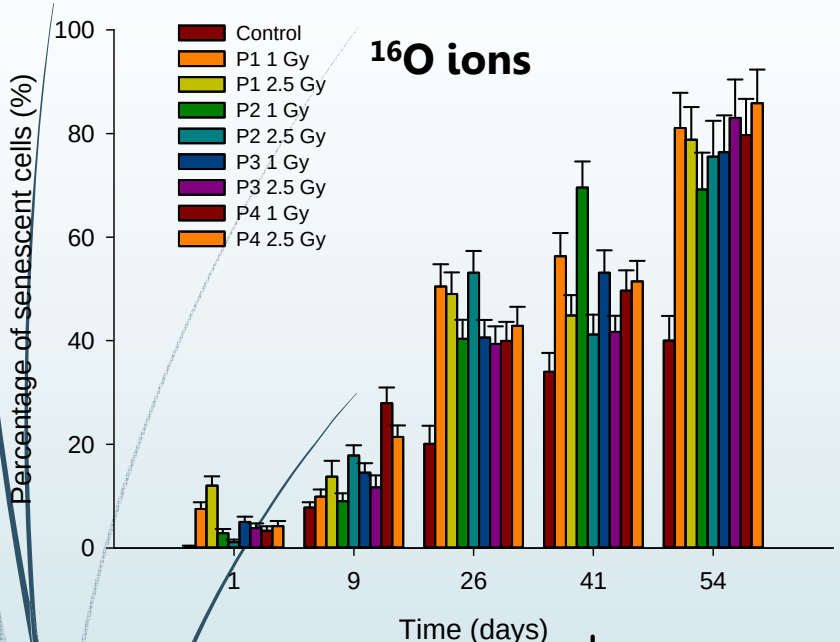
Sample positioning (arrows and squares): Positions (labeled as P1…P4) corresponded to estimated LET values of 68, 105, 409 and 769 keV/µm, respectively.

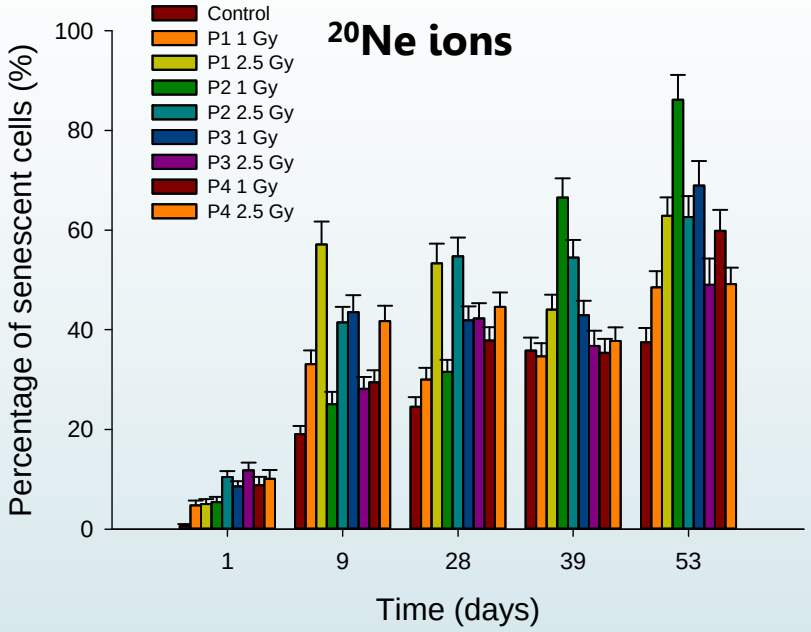
Towards «biological» Bragg curves

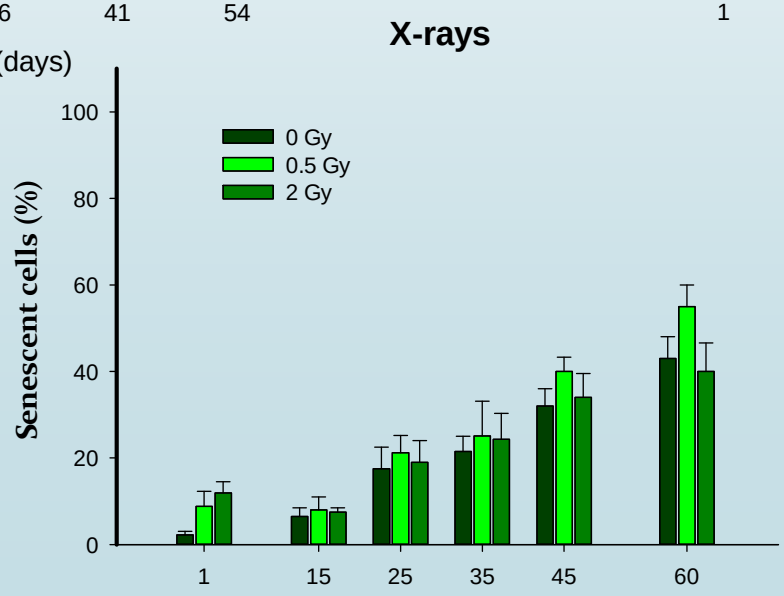


Positions P1, P3 and P4 were at LETs very close to those used for ^{16}O (115, 419 and 764 keV/μm) while P2 corresponded to 189 keV/μm

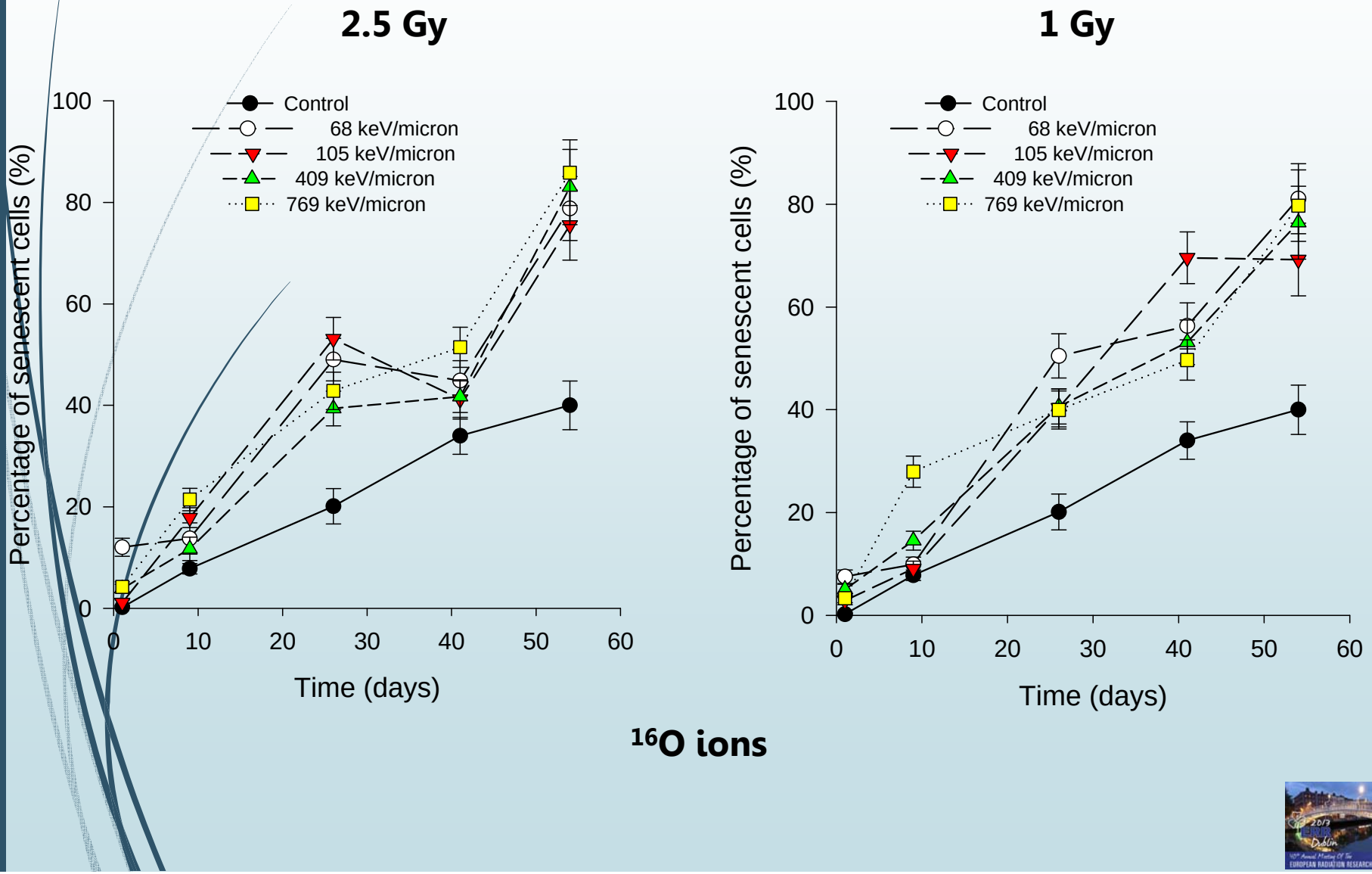
Towards «biological» Bragg curves





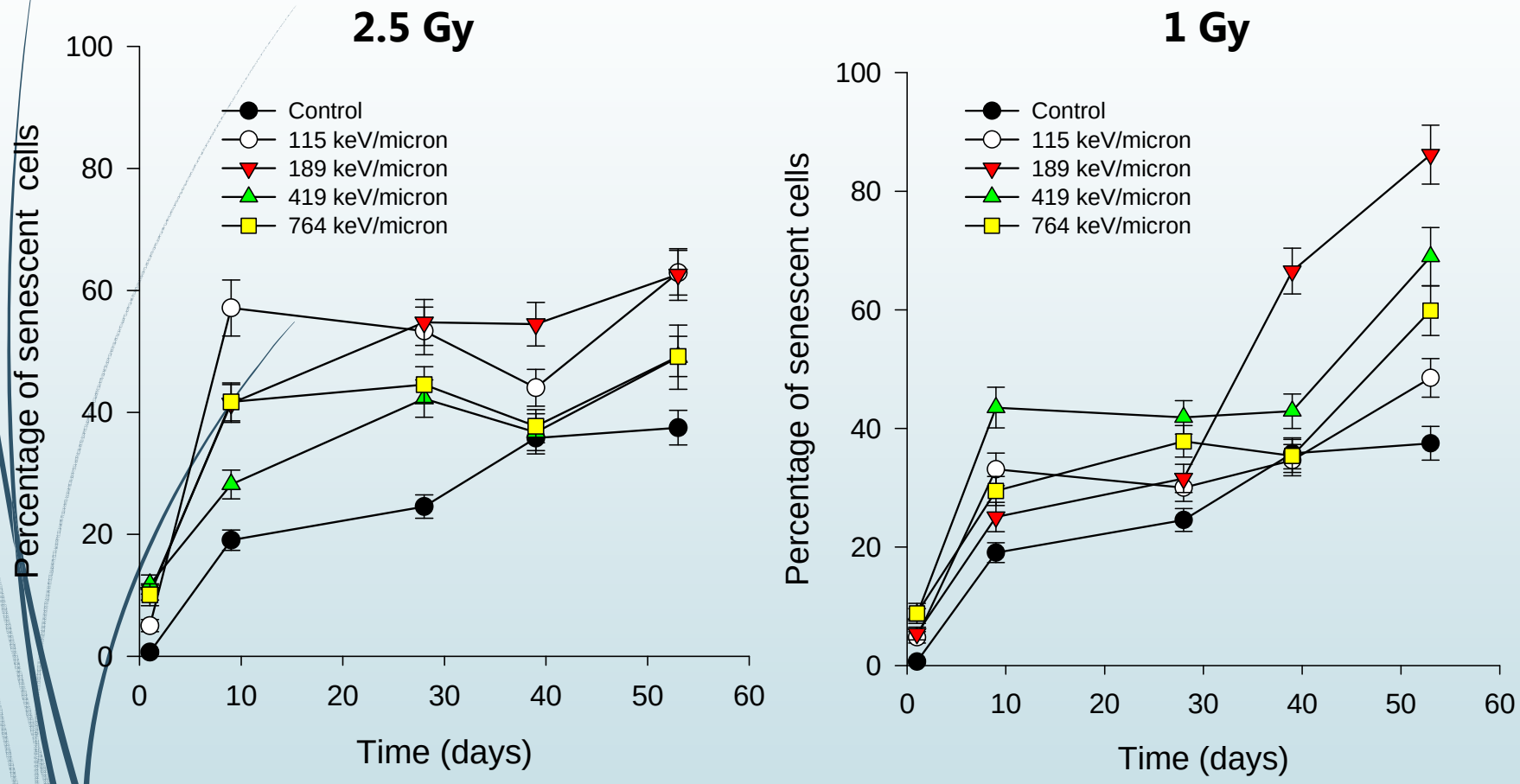


Towards «biological» Bragg curves



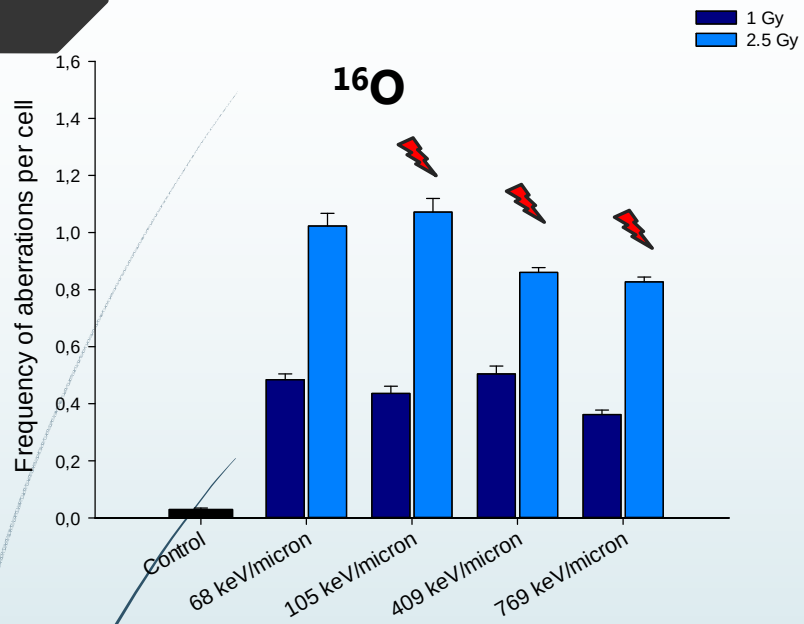
^{16}O ions

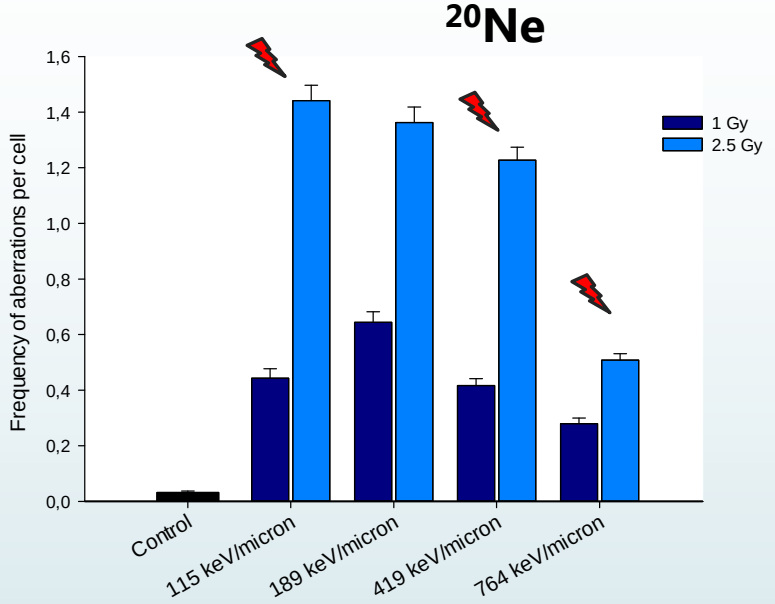
Towards «biological» Bragg curves

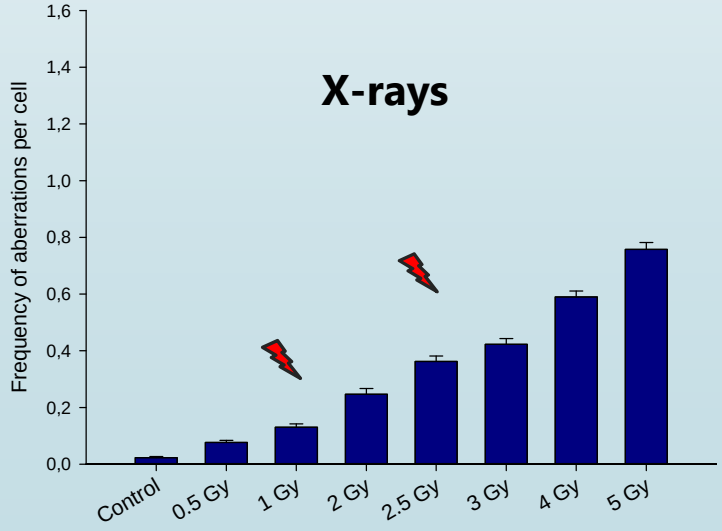


^{20}Ne ions

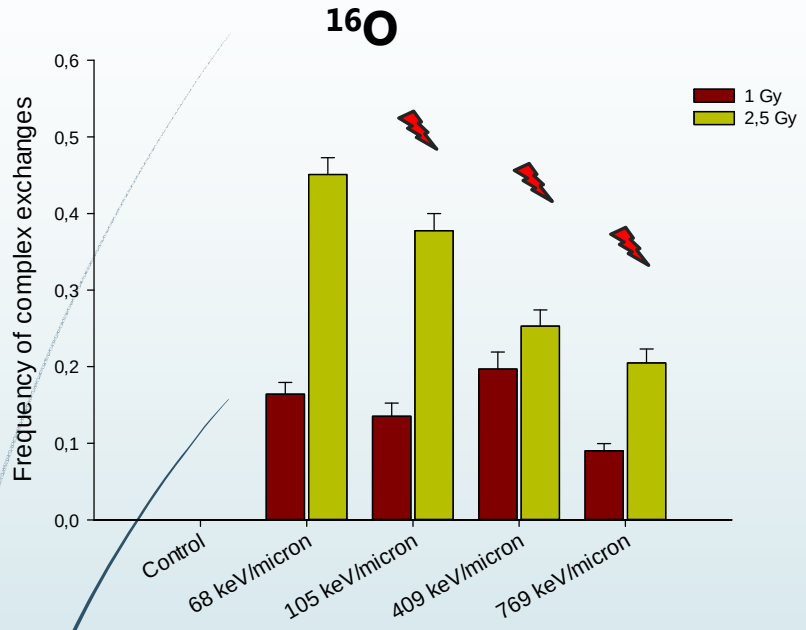
Towards «biological» Bragg curves

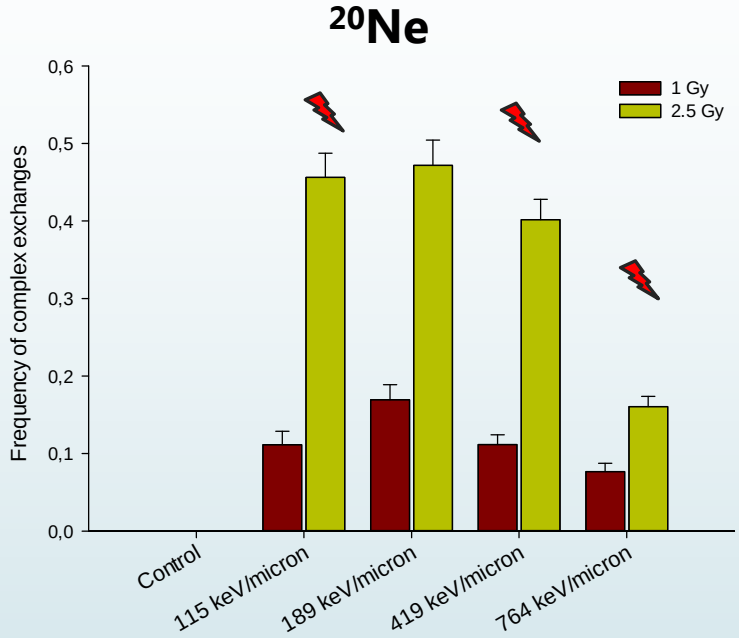


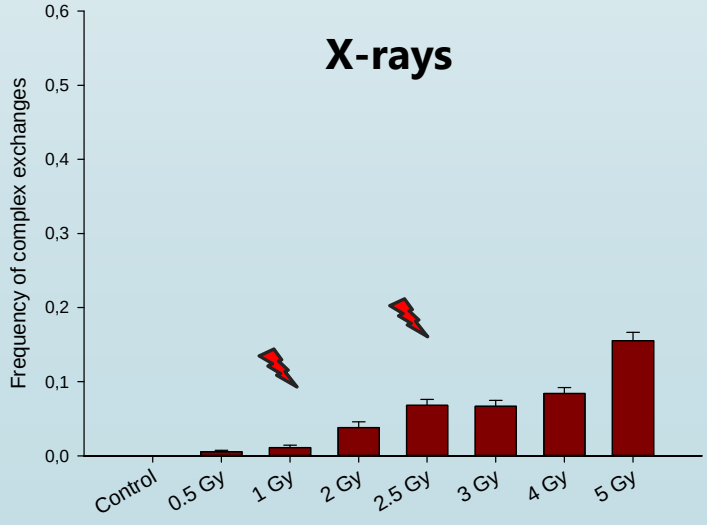




Towards «biological» Bragg curves







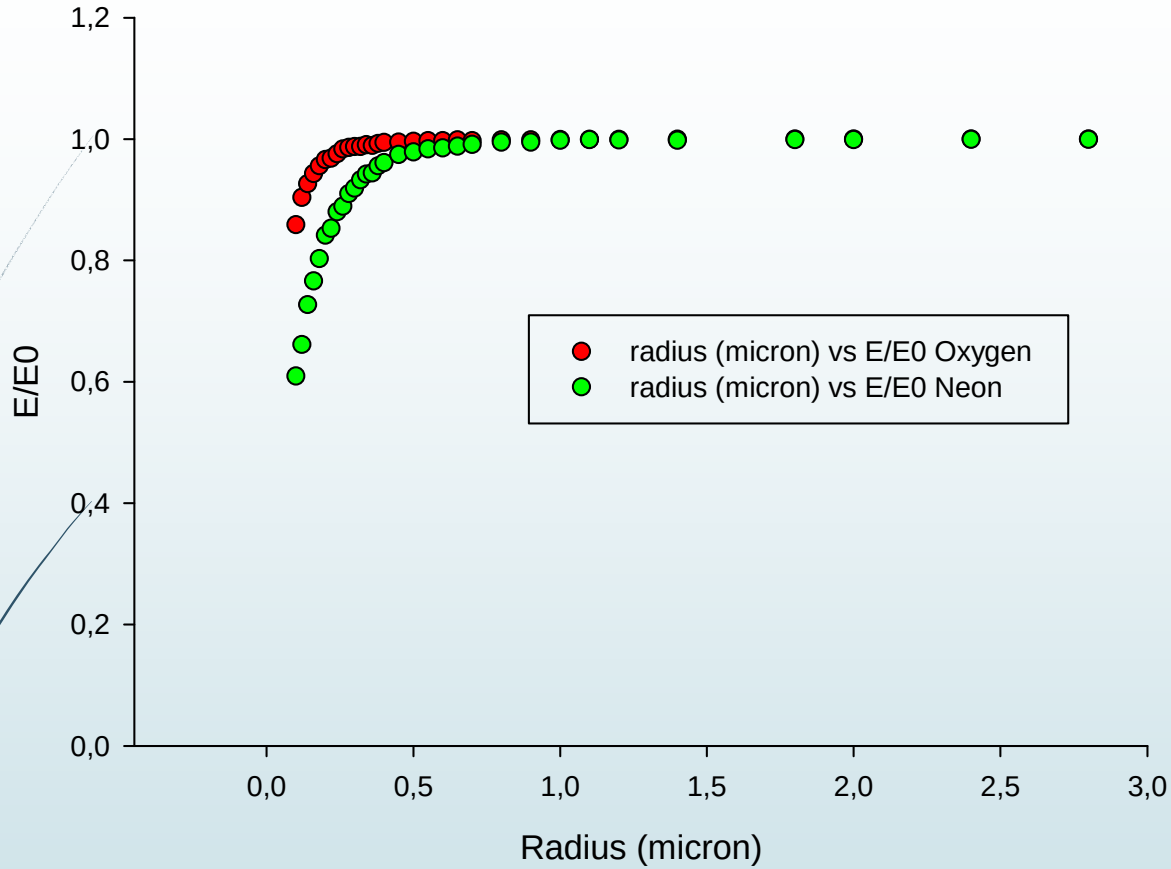
Outline

- Particle radiobiology
- Sub-lethal cytogenetic damage
- Towards «biological» Bragg curves
- Conclusions

Conclusions

- Charged particles are very effective at inducing cellular premature senescence, even at low doses
- Such induced senescence, at given LETs, depends on ion type and varies along the ion Bragg curve
- Chromosome damage induction also exhibits a dependence on ion type and on the ion penetration depth

energy oxygen vs. neon



- Monte-Carlo codes ought to incorporate track-structure features and sublethal biological data in modeling cellular response to charged particles.
- Implementing the newly developed module Geant_DNA to correlate ion-track structure with such results

Conclusions

- "The issue of **radiation risk during space exploration is unlikely to be solved by a simple countermeasure,** such as shielding or radioprotective drugs...The main uncertainties in risk-projection models will be reduced **only by improvement of basic understanding of the underlying biological processes and their disruption by space radiation**" *F.A.Cucinotta and M. Durante, Cancer risk from exposure to galactic cosmic rays: implications for space exploration by human beings, Lancet Oncol 2006; 7: 431–435 (2006)*
  - *B. Mukherjeea et al., Modulation of the DNA-damage response to HZE particles by shielding, Dna Repair 7 (2 0 0 8) 1717–1730, (2008)*
- "**Possible quantitative differences between low- and high-LET damage in causing telomere shortening or premature senescence are a concern for space radiation risk assessment**" *J.L Huff and F. A. Cucinotta, Human Research Program Requirements Document, HRP-47052, Rev.C, 2009.*

Conclusions

- "**The advent of radiotherapy using proton or heavy ion beam irradiations will inevitably lead to increases in SLGA-induction in vi**vo*" (Suzuki and Boothman, J. Radiat Res., 2008)*

- "**SIPS** is also induced **after exposure to lower doses of radiation**, which similarly has **consequences for understanding human cancer risk** to radiation exposure…Radiation-induced **non-targeted bystander (NTE) phenomenon is known to dominate at low radiation dos**es and to mediate a range of cellular effects…Thus, it is reasonable **to ask if there is a possible concordance between radiation-induced SIPS and SASP, and candidate mediators of NTE effect**s…. **Particularly at low doses future work needs to understand the relevance of radiation quality, dose and dose rate in initiating SIPS and the long-term tissue damage and pathological alterations** that may arise as a consequence" *R.J Sabin and R. M. Anderson, Cellular Senescence - its role in cancer and the response to ionizing radiation, Genome Integrity, 2, 2-9 (2011)*
  - ***Chronic exposure and cellular premature senescence (Bacq & Alexander Award Lecture, ERR2012 , M. Harms-Ringdahl)***

Acknowledgments

Gianfranco Grossi
Luigi Campajola
Ilaria Improta
Irene Magro
Francesca Margaret Perozziello
Giuseppe Signore
Gianfranca De Rosa
Fortuna De Martino

Pablo Cirrone
Francesco Romano

Kevin Prise
Giuseppe Schettino
Joy Kavanagh
Thomas Marshall
Pankaj Chaudhary

Marco Durante
Thilo Elsässer
Sylvia Ritter
Michael Scholz

Work partly funded by INFN grant MIMO-BRAGG

- SIPS is also induced after exposure to lower doses of
- radiation [125,130,132,133] which similarly has consequences
- for understanding human cancer risk to radiation
- exposure, but this time within the context of SIPS
- in normal tissue after e.g. diagnostic exposures. For
- instance it is well established that radiation induces
- damage in cells that are not directly irradiated but
- which are in communication with irradiated cells. This
- radiation-induced non-targeted bystander (NTE) phenomenon
- is known to dominate at low radiation doses
- and to mediate a range of cellular effects such as DNA
- damage [134,135], cell death [136], cell proliferation,
- adaptive protective effects and malignant transformation
- [75,137-141]. To date, such NTE have been observed in
- microbeam-irradiated human tissue [141,142], in vivo
- animal models [143-145] and interestingly, in cells cultured
- in both non-irradiated tumour and senescent cell
- conditioned medium [14,75].
- Thus, it is reasonable to ask if there is a possible concordance
- between radiation-induced SIPS and SASP,
- and candidate mediators of NTE effects

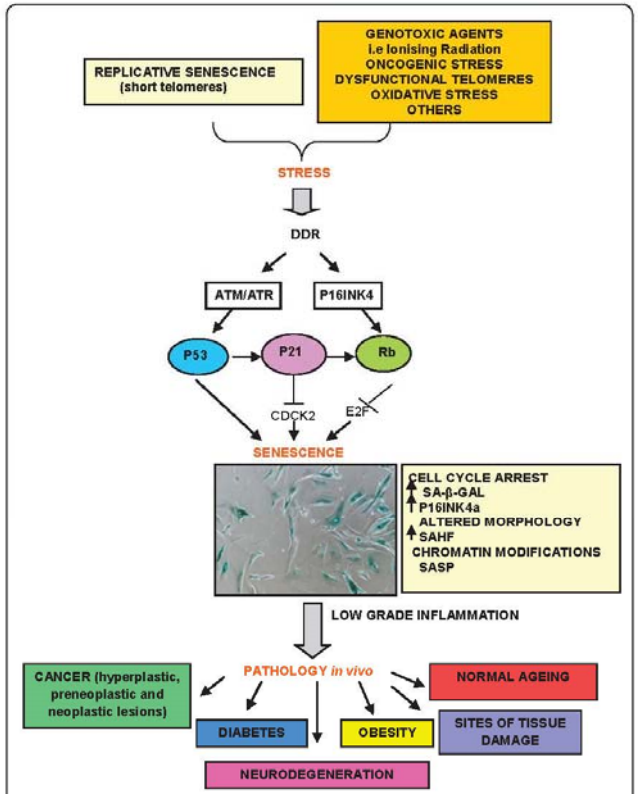
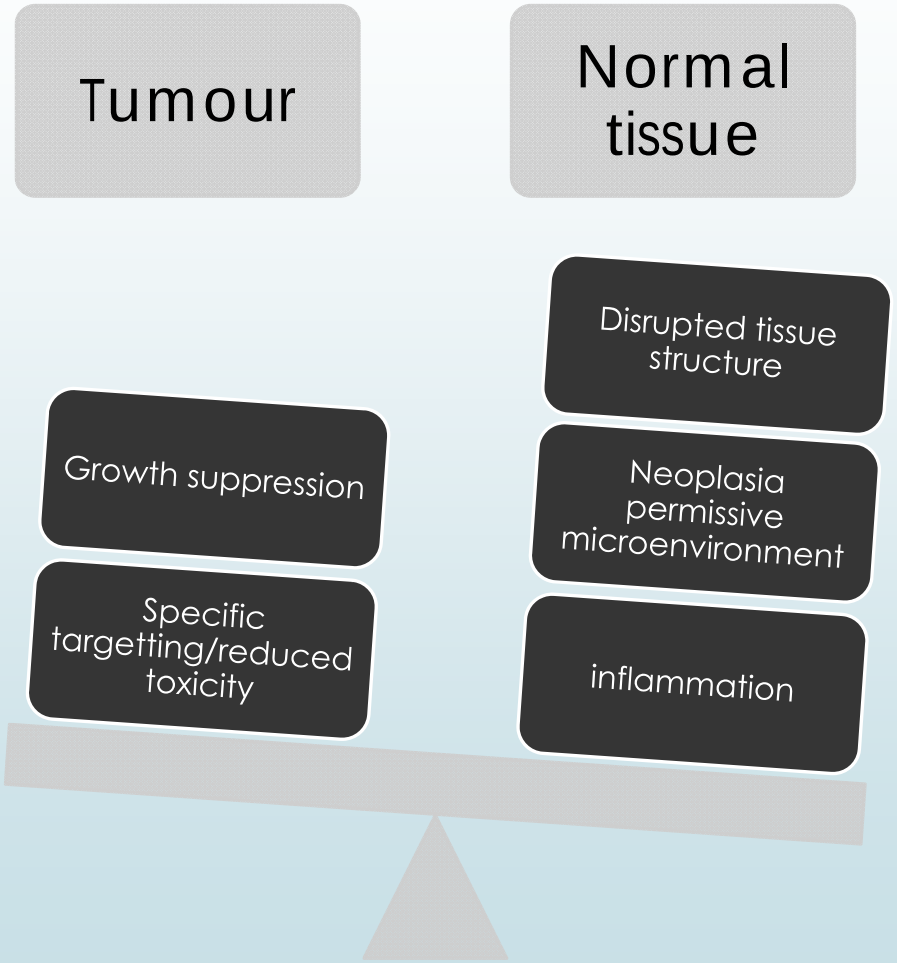


Figure 1 Scheme highlighting initiating and molecular mediators of cellular senescence. The senescent phenotype includes expression of SA-β-galactosidase (SA-β-gal), increased expression of p16INK4a leading to cell cycle arrest and an increase in the secretion of pro-inflammatory factors termed as senescence-associated secretory phenotype (SASP). Senescent cells have been observed in normal ageing cells and in cells/tissues of various age-related pathologies.

- Further, cells undergoing SIPS reportedly secrete factors inhibiting or promoting tumour proliferation (Senescence–Associated Secretory Phenotype). A well-known cancer biomarker, CAs may lead to genomic instability and transformation. Results show that cytogenetic damage does vary along ion path and, for similar LETs, between ion species. Hence, Monte-Carlo codes ought to incorporate track-structure features and sublethal biological data in modeling cellular response to charged particles.

Implications

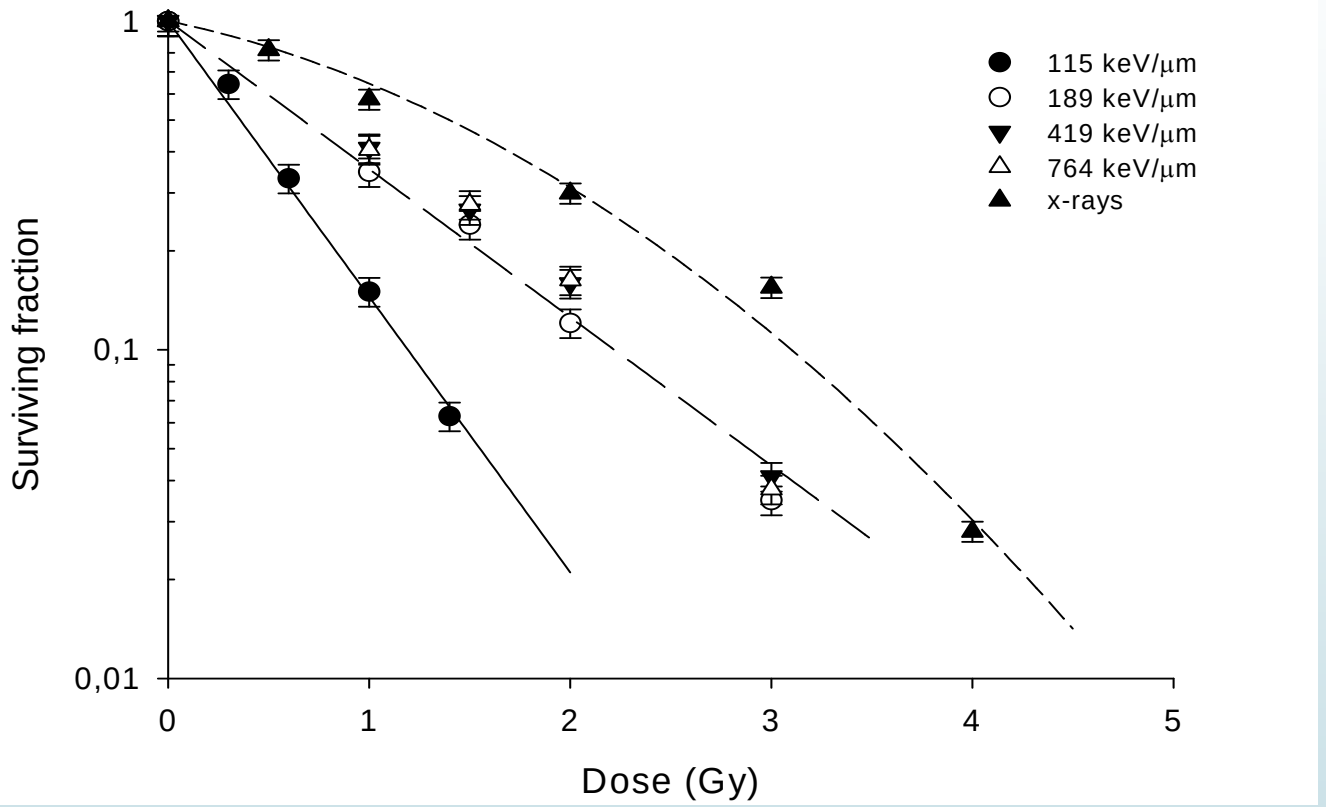
- Ion-induced senescence is both an acute and persistent response
- Significant deviations from predicted radiobiological behaviour at very high LETs
  - Not only do ions mantain higher cell killing abiliy than photons, but also induce more effectivecly sublethal long-term effects, and possibly carry higher carcinogenic risk since higher initial yield of aberrations translates in less long-term senescence
  - **This warrants caution (space missions, healthy tissue complication in heavy ion radiotherapy) and revisitation of particle radiation modellization**


Tumour
Normal tissue
Growth suppression
Specific targetting/reduced toxicity
Disrupted tissue structure
Neoplasia permissive microenvironment
inflammation

Preliminary conclusions/2

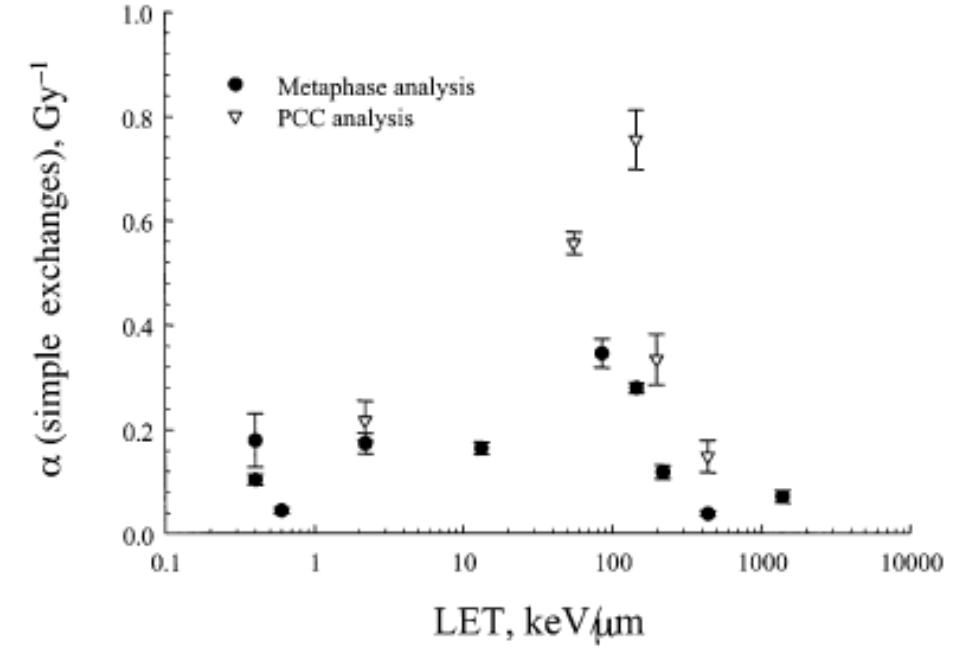
- ^{20}Ne induces higher aberration frequency than ^{16}O at 100-400 keV/μm
- This is true also for complex-type damage
- ^{20}Ne causes more fragmentation at all LETs

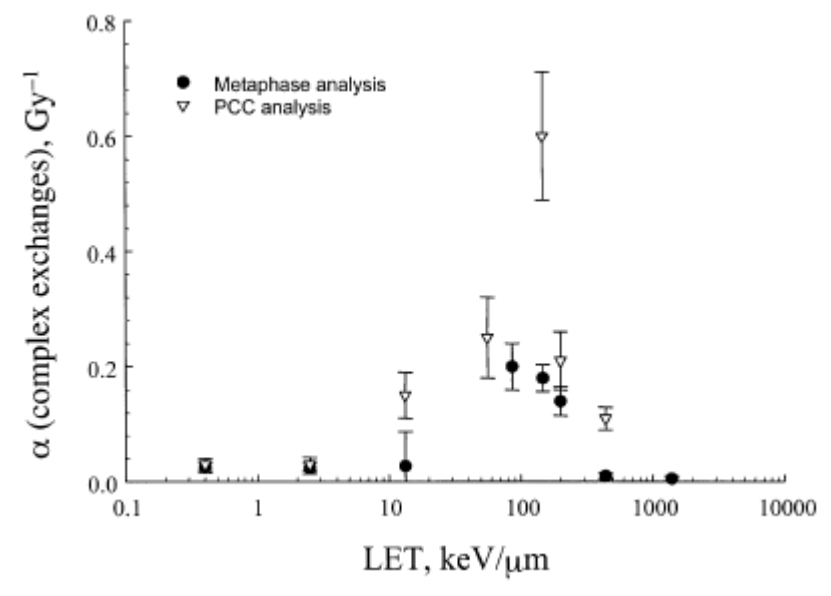
Insights from cell killing



Clonogenic survival curves for AG01522 cells after ^{20}Ne ion irradiation; survival after x-rays is also shown as a comparison.

RBE for different aberration types





α (Gy^{-1}) vs. LET for different light and heavy ions

Human lymphocytes exposed in G_0-phase

Data from George *et al. Radiat. Res.* **160** (2003) 425

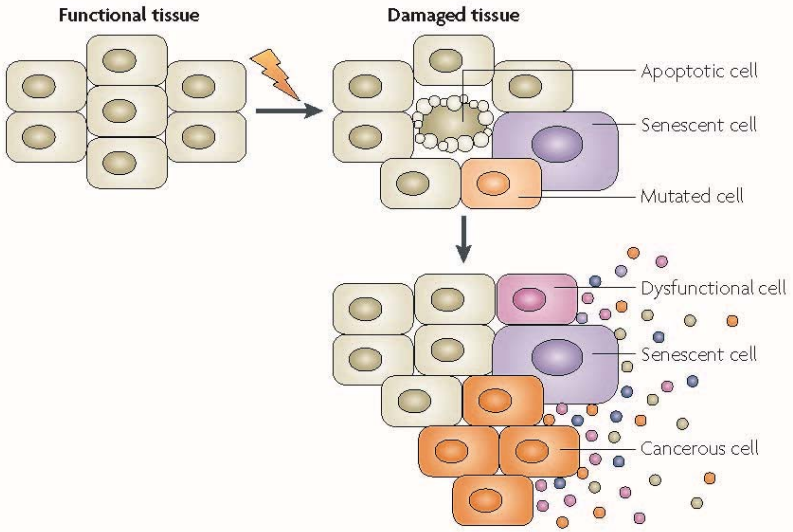
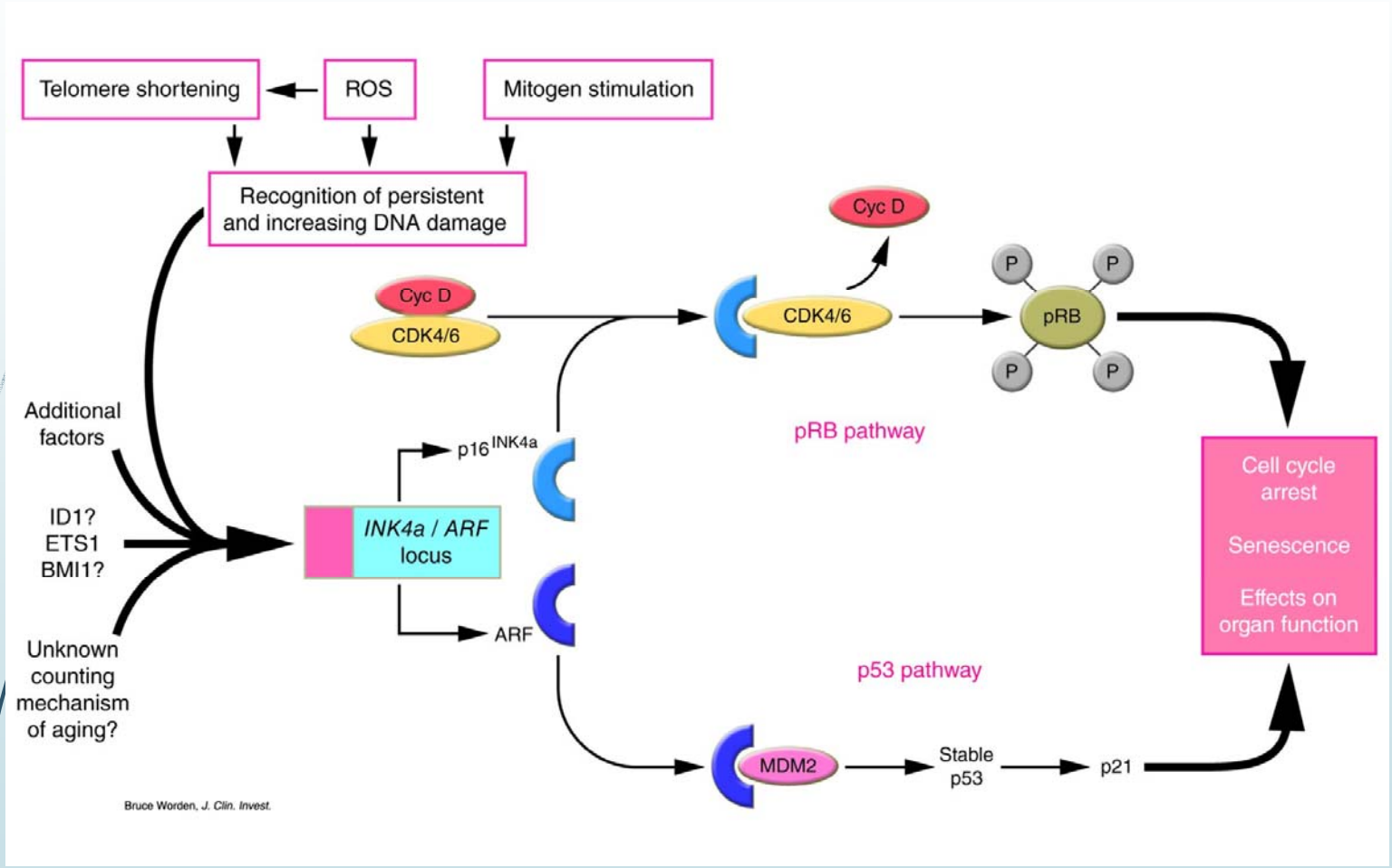


Figure 5 | **Potential deleterious effects of senescent cells.** Damage to cells within tissues can result in several outcomes. Of course, the damage may be completely repaired, restoring the cell and tissue to its pre-damaged state. Excessive or irreparable damage, however, can cause cell death (apoptosis), senescence or an oncogenic mutation. The division of a neighbouring cell, or a stem or progenitor cell, usually replaces apoptotic cells. Cell division, however, increases the risk of fixing DNA damage as an oncogenic mutation, leaving the tissue with pre-malignant or potentially malignant cells. Senescent cells, by contrast, may not be readily replaced; in any case, their number can increase with age. Senescent cells secrete various factors that can alter or inhibit the ability of neighbouring cells to function, resulting in dysfunctional cells. They can also stimulate the proliferation and malignant progression of nearby premalignant cells. Therefore, an accumulation of senescent cells can both compromise normal tissue function and facilitate cancer progression.


Telomere shortening
ROS
Mitogen stimulation
Recognition of persistent and increasing DNA damage
Cyc D
CDK4/6
Cyc D
CDK4/6
P
P
pRB
P
P
pRB pathway
Additional factors
ID1?
ETS1
BMI1?
Unknown counting mechanism of aging?
p16INK4a
INK4a / ARF locus
ARF
Cell cycle arrest
Senescence
Effects on organ function
p53 pathway
MDM2
Stable p53
p21
Bruce Worden, J. Clin. Invest.