Ruolo delle nanoparticelle d'oro nella radioterapia con fasci esterni per la cura del tumore al seno

A. Tudda(1, 2), G. Mettivier (1, 2), G. Nicolini (3, 4), E. Donzelli (3, 4), S. Semperboni (3, 4), M. Bossi (3, 4), G. Cavaletti (3, 4), R. Castriconi (5, 6), P. Mangili (5, 6), A. Sarno (2), A. del Vecchio (5, 6), P. Russo (1, 2)

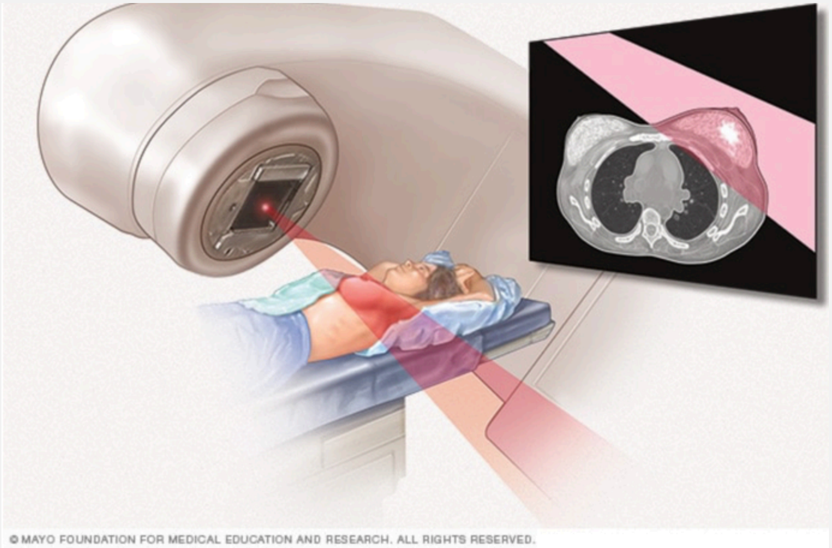
(1) Università di Napoli Federico II, Dipartimento di Fisica "Ettore Pancini", Napoli,
(2) INFN Sezione di Napoli,
(3) Unità Sperimentale Neurologica, Scuola di Medicina e Chirurgia, Università di Milano-Bicocca, Monza,
(4) INFN Sezione di Milano-Bicocca, Milano,
(5) Fisica Medica, IRCCS Istituto Scientifico San Raffaele, Milano
(6) INFN Sezione di Milan

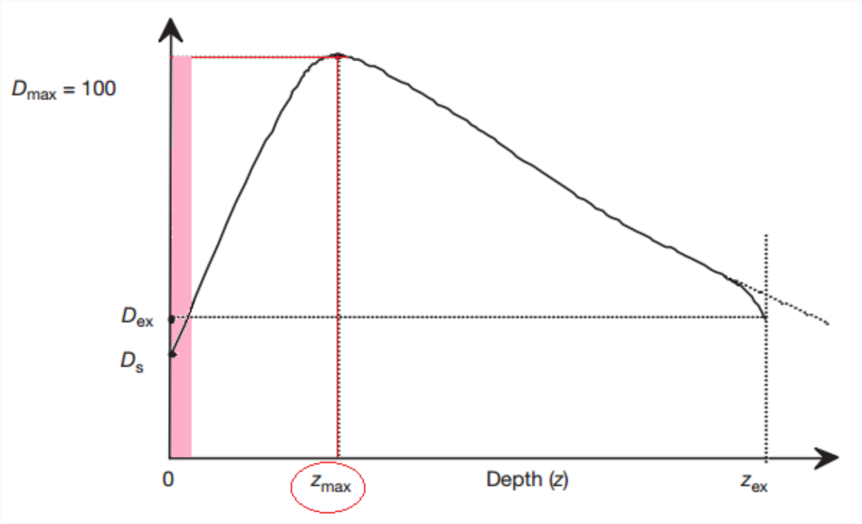
Alessia Tudda – 16/09/2020

Conventional Radiotherapy for Breast Cancer

- External Breast Radiation Therapy (**EBRT**) is the most common approach for breast cancer treatment

MV X-Rays (6 MV)





- Dose Sparing
- Maximum of the dose in tumour volume
- Dose to Organ At Risk (OAR) and Normal Tissue

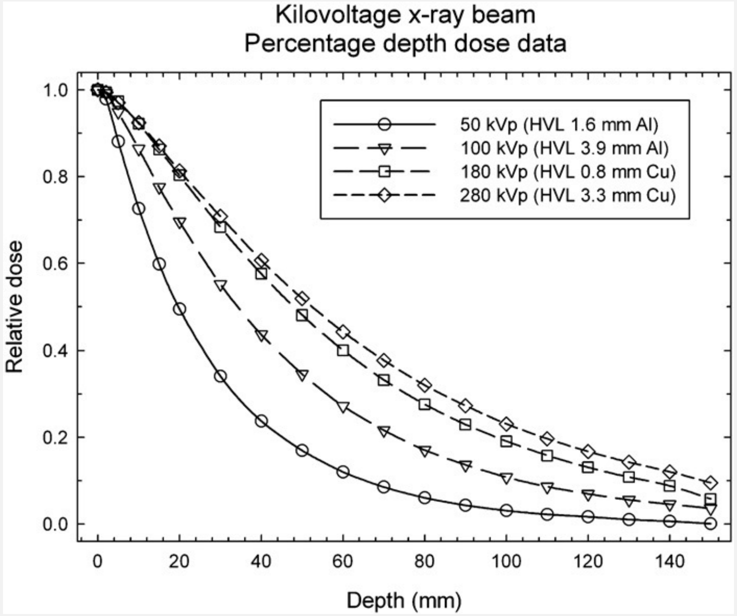
↓

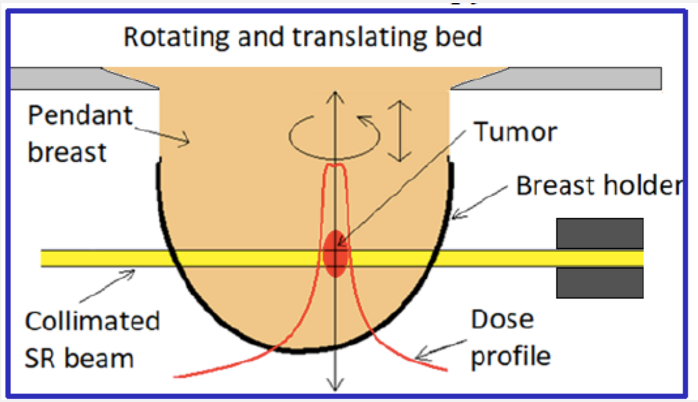
metastases, relaps, toxicity to Normal Tissue

- Necessity of new RT approach that increasing dose to the tumour while sparing NT and OAR

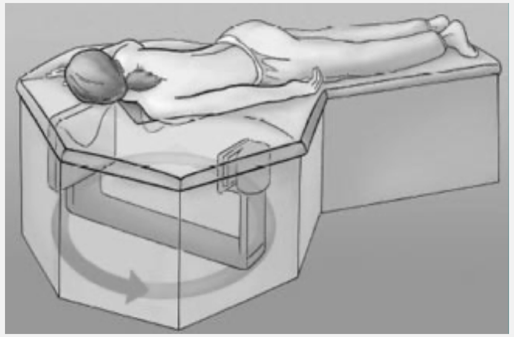
https://www.mayoclinic.org/testsprocedures/radiation-therapy/about/pac-20385162

SR^{3}T project: a new EBRT approach with kV energies





Kilovoltage Rotational External Beam RT



***S**ynchrotron **R**adiation **R**otational **R**adio**T**herapy* (comprises INFN branches of Naples, Ferrara, Milano, Cagliari, Roma)

a) Why Synchrotron Radiation? →
- Higher photons flux than orthovoltage photons flux
- Monoenergetic **kV** beams

b) Why Rotational? → due to the **principle of rotational summation of the absorbed dose**

RATIONALE → Selective breast Radiotherapy (**kV SR radiation** + **Au nanoparticles**)

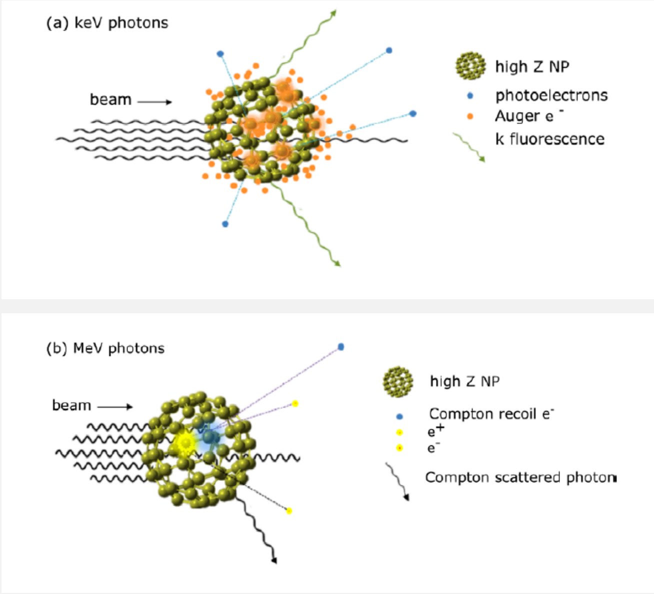
F. Di Lillo et al., *Synchrotron radiation external beam rotational radiotherapy of breast cancer: proof of principle*, Journal of synchrotron radiation, **25**, 857-868, (2018)
F. Di Lillo et al., *Towards breast cancer rotational radiotherapy with synchrotron radiation*, Physica Medica, **41** , 20–25, (2017).

Medical Physics Research Laboratory

Metal Nano Enhancers in RT – State of the Art

- Since early 90's it was investigated the possibility of utilizing METAL NANO ENHANCERS
  - High Z
  - Size of 1-100 nm
  - lead to radiosensitization enhancement in cancer therapy where they are localized

- Several metallic elements have demostrated (*in vivo* and *in vitro*) efficiency in radiosensitization (Gd, Hf, Pt, Au and others)



- Most relevant results have been shown with synergic action between Metal Nano Enhancers + X-rays (80 keV – 25 MeV)

  → Metal Nano Enhancers + Ion Therapy (protons)

Kuncic Z. and Lacombe S., *Nanoparticle radio-enhancement: principles, progress and application to cancer treatment*, Physics in Medicine and Biology, 63 (**2**), 1-27, (2018)
https://www.inc.uam.es/wp-content/uploads/radiation_effects.pdf
Liu Y., Zhang Z., Li F.,Metal-based NanoEnhancers for Future Radiotherapy: Radiosensitizing and Synergistic Effects on Tumor Cells, Theranostics, 1, (8), 1824-1849, (2018)

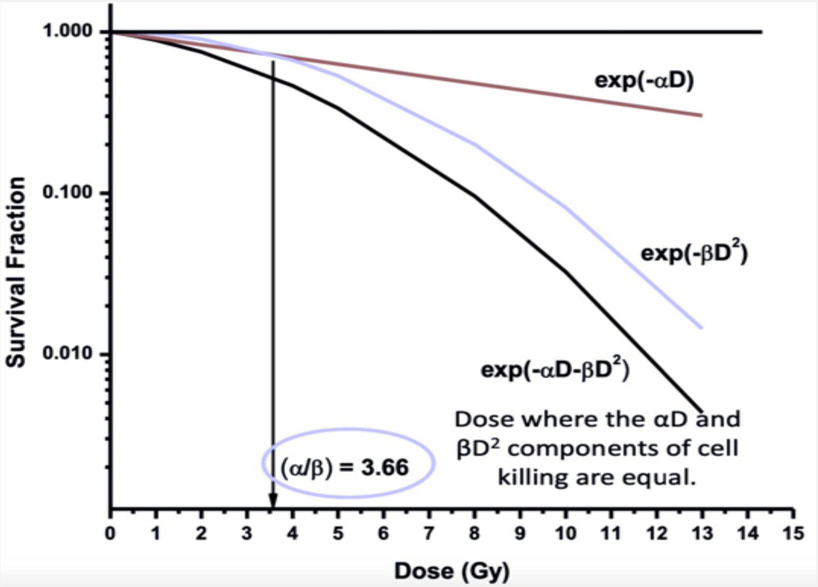
Dose Enhancement Measurements

- A damage quantification (*in vivo* and *in vitro*) of the effect of Metal Nano Enhancer is expressed by:

$$\textbf{Dose Enhancement Factor} = \left.\frac{D_{without\ NP}}{D_{NP}}\right|_{specific\ level\ of\ biological\ effect}$$

- Gold Standard for the evaluation of radio-induced damage *in vitro* is the :

CLONOGENIC ASSAY



$$SF = \frac{colonies\ counted}{cells\ seeded \times \left(^{PE}/_{100}\right)}$$

$$PE = \frac{number\ of\ colonies\ counted}{number\ of\ cells\ seeded}\ X\ 100$$

Linear Quadratic Method

$$SF = e^{-\alpha D - \beta D^2}$$

α → Low Doses
β → High Doses

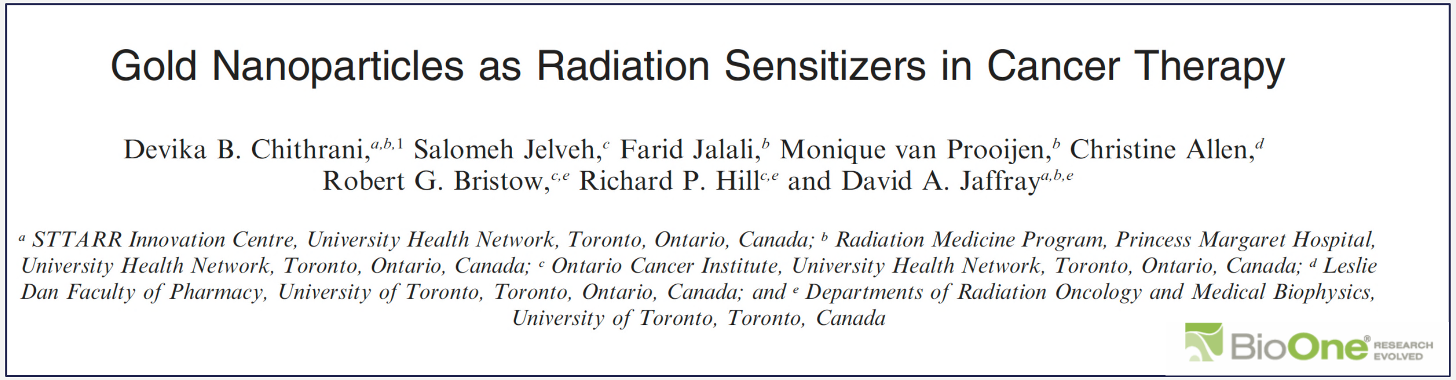
Kuncic Z. and Lacombe S., *Nanoparticle radio-enhancement: principles, progress and application to cancer treatment*, Physics in Medicine and Biology, 63 (**2**), 1-27, (2018)
https://www.inc.uam.es/wp-content/uploads/radiation_effects.pdf

Gold as Nano Enhancers in conventional RT

- Gold appears to be the favorite Metal Nano Enhancers:
  - Easy to fabricate in nanometers sizes
  - Non toxic
  - Biocompatible characteristics
  - Well absorbed into systemic circulations

AuNPS

- Several and relevant studies have been demostrated a radiosensitation effect with MV X-rays energies and AuNps

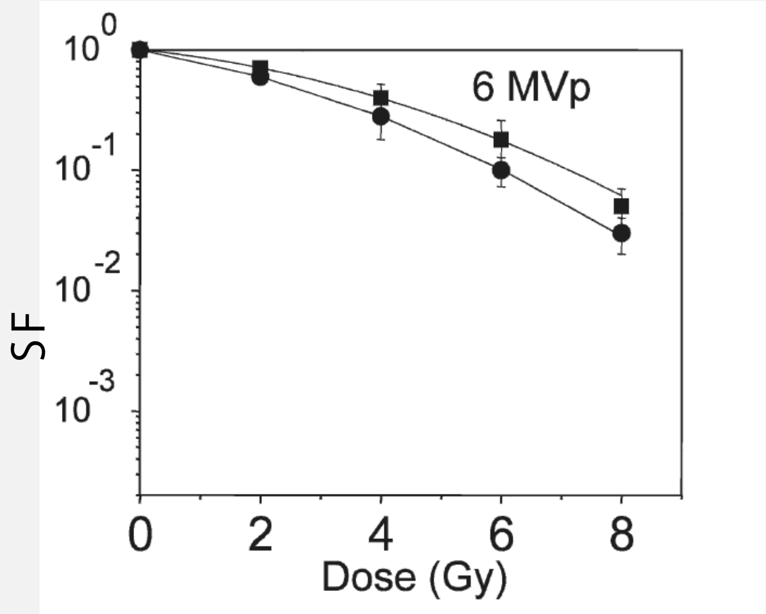


Gold Nanoparticles as Radiation Sensitizers in Cancer Therapy

Devika B. Chithrani,[a,b,1] Salomeh Jelveh,[c] Farid Jalali,[b] Monique van Prooijen,[b] Christine Allen,[d] Robert G. Bristow,[c,e] Richard P. Hill[c,e] and David A. Jaffray[a,b,e]

[a] STTARR Innovation Centre, University Health Network, Toronto, Ontario, Canada; [b] Radiation Medicine Program, Princess Margaret Hospital, University Health Network, Toronto, Ontario, Canada; [c] Ontario Cancer Institute, University Health Network, Toronto, Ontario, Canada; [d] Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada; and [e] Departments of Radiation Oncology and Medical Biophysics, University of Toronto, Toronto, Canada

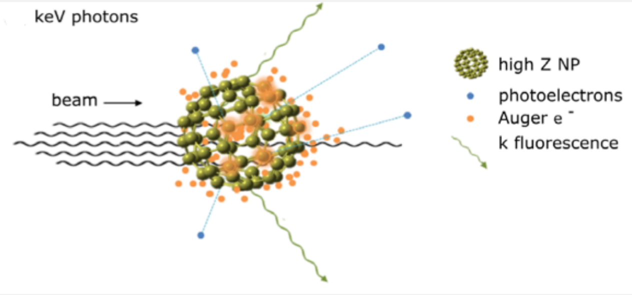
BioOne RESEARCH EVOLVED



Squares: treatment only (6 MV photon beam) on HeLa cells;
Circles: irradiation of 6 MV photon beam after injection of $3x\ 10^{-3}$ % (1 ~ nm) of 50 nm GNPs on HeLa cells

Kwatra D, Venugopal A, Anant S. Nanoparticles in radiation therapy: a summary of various approaches to enhance radiosensitization in cancer. Transl Cancer Res 2013;2(4):330-342

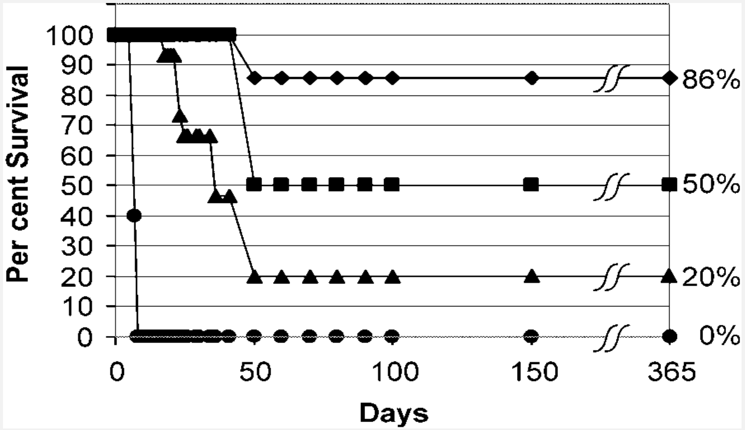
Gold as Nano Enhancers – kV energies



- It could be preferable using kV energies $\sigma_{PE} \propto Z^3 / E^3$

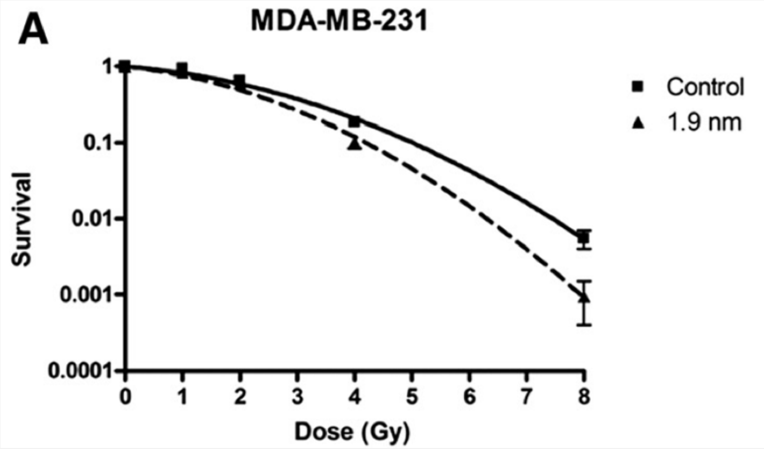
- There are in vitro and in vivo experiments revealing dose enhancement due to synergic interaction between AuNPs and kV energies

In vivo experiment: Mice survival after treatments of subcutaneous EMT-6 tumours



Circles: no treatment, gold only (1.35 g Au/kg, no irradiation);
Triangles: irradiation only (26 Gy, **250 kVp**);
Squares: irradiation after intravenous injection of 1.35 g Au/kg ;
Diamonds: irradiation after 2.7 g Au/kg intravenous injection

In vitro experiment: Radiation dose response curves for MDA-MB-231 cells



Triangles: irradiation with **225 kVp** X-rays of cells pretreated with 500 μg/ml AuNPs
Squares: irradiation only

Taggart L. E.et al., *The role of mitochondrial function in gold nanoparticle mediated radiosensitisation*. Cancer Nanotechnology, 5(**5**) , 1-12, (2014)
Herold DM, et al., *Gold microspheres: a selective technique for producing biologically effective dose enhancement*, Int J Radiat Biol., **76**, 1357-64, (2000)

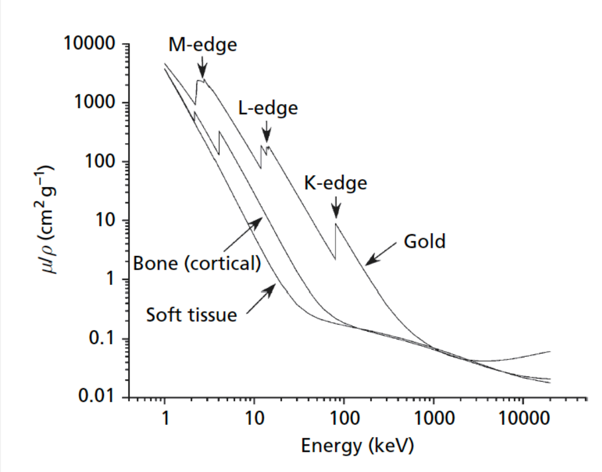
Medical Physics Research Laboratory

Gold as Nano Enhancer and kV X-rays - choice of energy

- Choice of prefereble energy is a crucial issue, a good choice could be above the K-edge (80.7 keV)

- Primary photoelectric effect products are Photoelectrons

$$E_{photoelectron} = E_0 - E_b$$



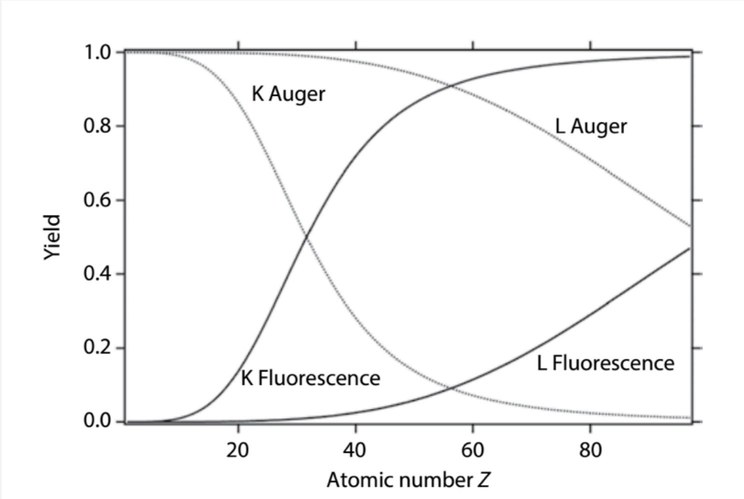
  i. Energy ~ K-edge Energy → Low energergetic photoelectrons

  ii. Energy ≳ K-edge Energy (~130 KeV) →
     - Photoelectrons travel ~ 40 µm (4-8 cells)
     - Photoelectric cross section decreases (3.7 times)

- Secondary photoelectric effect products could be

  - Auger electron
    - ❖ Double Streand Break
    - ❖ Distance ~ few nm

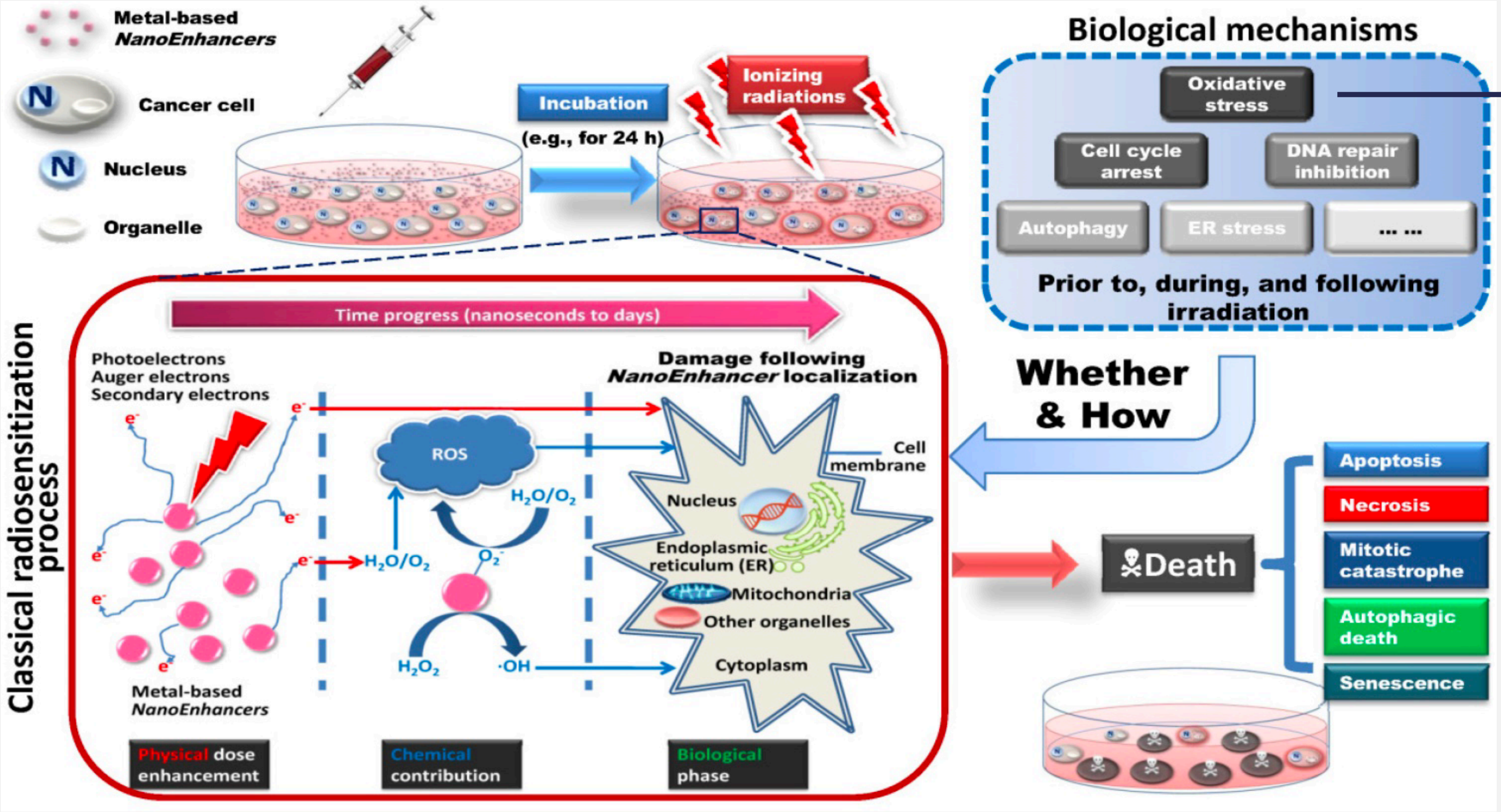
  - K fuorescence
    - ❖ Single Streand Break
    - ❖ Distance up to cm



R. Menk, "Basic Physics of X- ray interaction in matter". In: "Handbook of X-ray Imaging Physics and Technology" (P. Russo, Ed.) , p. 17, 20 CRC Press. 2018.
Hainfeld J.F., Dilmanian F. A., Slatkin D.N., Smilowitz H., Radiotherapy enhancement with gold nanoparticles, Journal of Pharmacy and Pharmocology, 60 (8), 977-985 (2007)

Biophysical and biochemical mechanisms of metal nanoenhancers

- Presence of AuNPs could induce stress to cells. Enhanced cell killing effect resulting from synergic action of AuNPs and photon is due to Physical, Chemical, Biological effects



·OH production

- DNA damage
- Mythocondrial damage

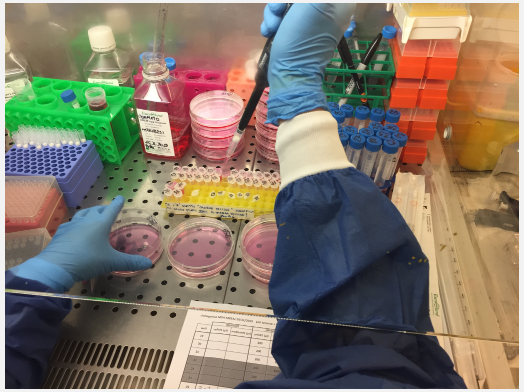
Liu Y., Zhang Z. et al., *Metal-based NanoEnhancers for Future Radiotherapy: Radiosensitizing and Synergistic Effects on Tumor Cells*, Theranostics, 1, (8), 1824-1849, (2018).

Dose Enhancement measurements

Medical Physics Research Laboratory

UNIVERSITA' DEGLI STUDI DI MILANO BICOCCA

OSPEDALE SAN RAFFAELE



Clonogenic Assay

↓

Dose Response curve: Survival Fraction vs Dose with and without AuNPs

- 100 kV → MDAMB231 line cells + 15 nm AuNPs Aurovist™
- 190 kV → MDAMB231 line cells + 15 nm AuNPs Aurovist™

→ Orthovoltage X-ray tube at University Milano, Bicocca

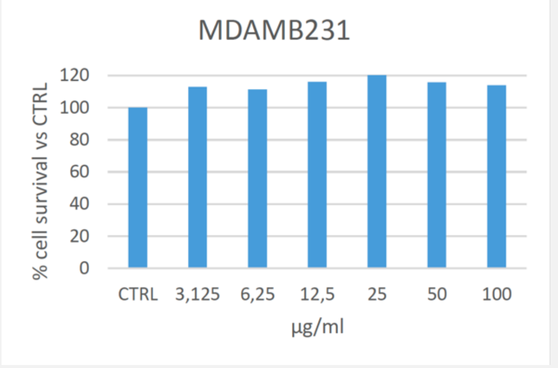
- 6 MV → MDAMB231 line cells + 15 nm AuNPs Aurovist™ → Linac (clinical accelerator) at San Raffaele Hospital (Milano)

$$DEF = \left.\frac{D_{NO\ AuNP}}{D_{AuNP}}\right|_{50\%\ SF}$$

$$SF\ ratio = \left.\frac{SF_{No\ AuNP}}{SF_{AuNP}}\right|_{Gy}$$

Radiobiology for the radiologists", P. Hall, (p. 35), ISBN 10: 047132406X

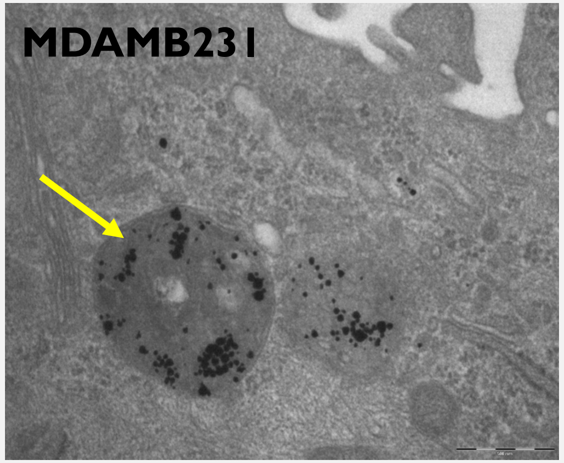
Preliminary experiments

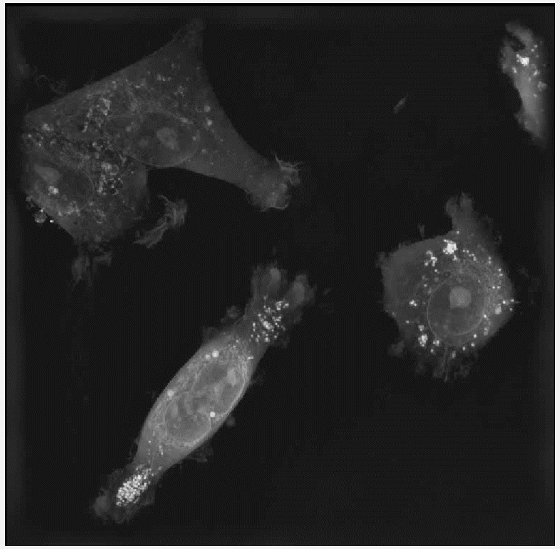


- *Measures of toxicity* → SulfoRhodamine B (SRB) assay

% cell survival vs Control > 83 % → **No toxicity AuNPs induced**

- *Measures of internalization of 15 nm AuNPs* → TEM image analysis





i. AuNPs were internalized by MDAMA231 forming clusters 24 h after internalization
ii. Presence of AuNPs in MDAMB231 6 h after injection
iii. No AuNP is in the nucleus

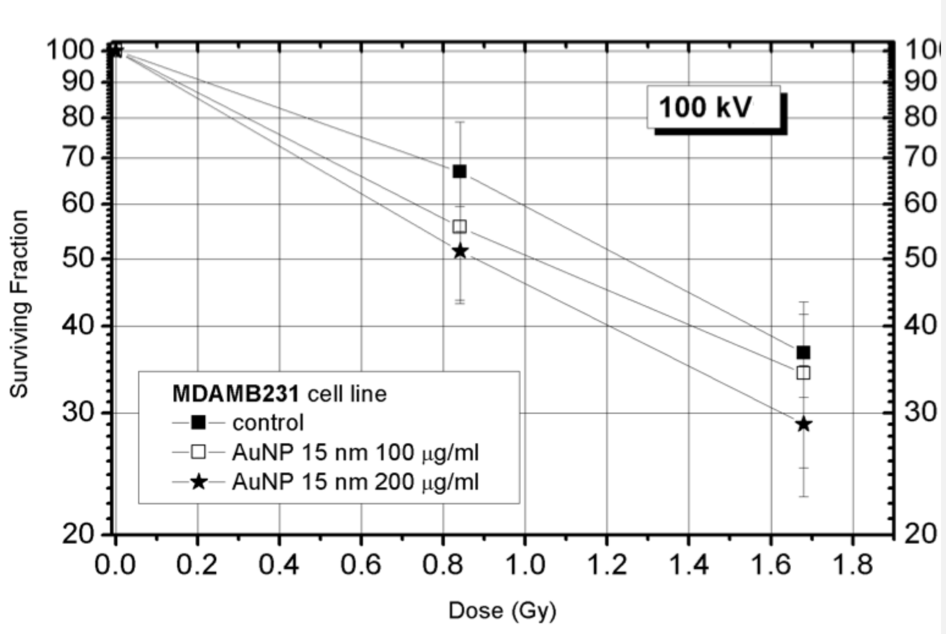
- *Atomic Absorption Spectroscopy measures of 15 nm AuNPs*

Experimental Neurology Unit, school of Medicine and Surgery, Università Milano Bicocca

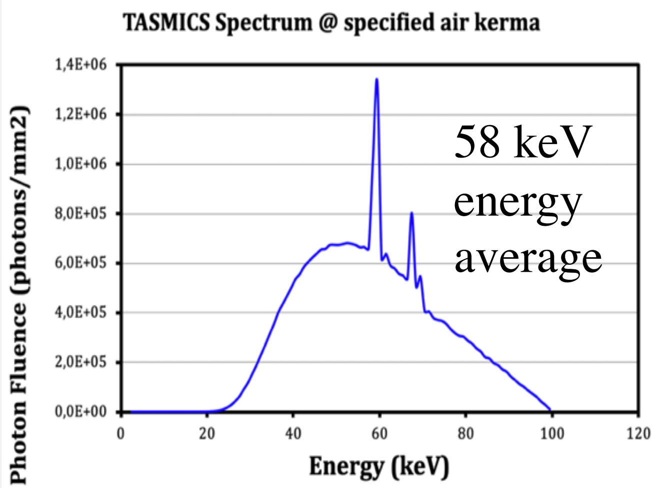
100 kV measurements

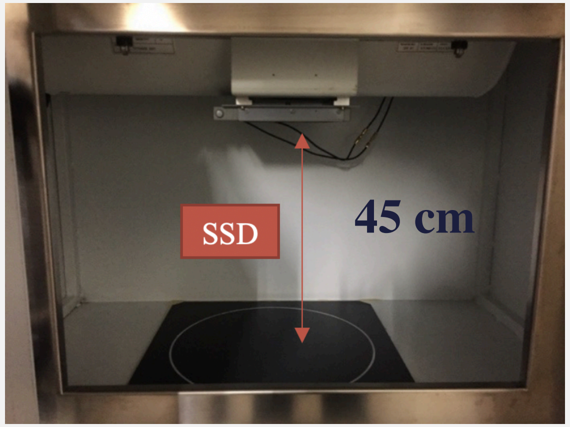
MDAMB231
irradiation at **1** and **2** Gy (nominal dose) →
- **0 μg/ml (cntrl)**
- **100 μg/ml**
- **200 μg/ml**

} **15 nm AuNP**



(results of three measurement)





$$SF\ ratio = \left.\frac{SF_{No\ AuNP}}{SF_{AuNP}}\right|_{Gy}$$

CELL LINE	X-ray tube potential	AuNP CONCENTRATION	AuNPs size	SURVIVAL FRACTION RATIO AT 1 Gy (nominal dose)	SURVIVAL FRACTION RATIO AT 2 Gy (nominal dose)
MDAMB231	100 kV	100 μg/ml	15 nm	**1.2±0.3**	**1.0±0.3**
MDAMB231	100 kV	200 μg/ml	15 nm	**1.3±0.2**	**1.3±0.3**

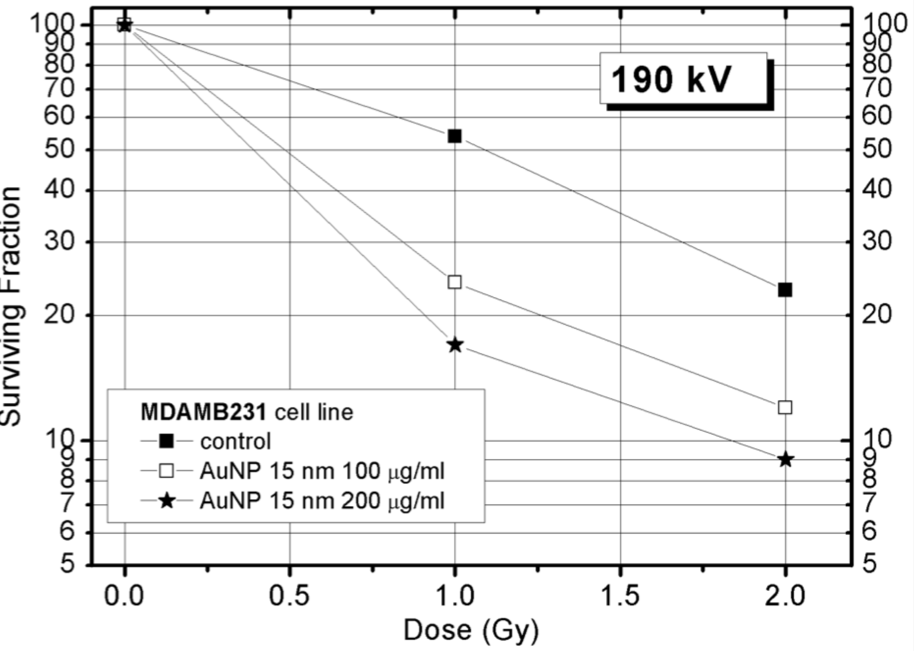
Medical Physics Research Laboratory

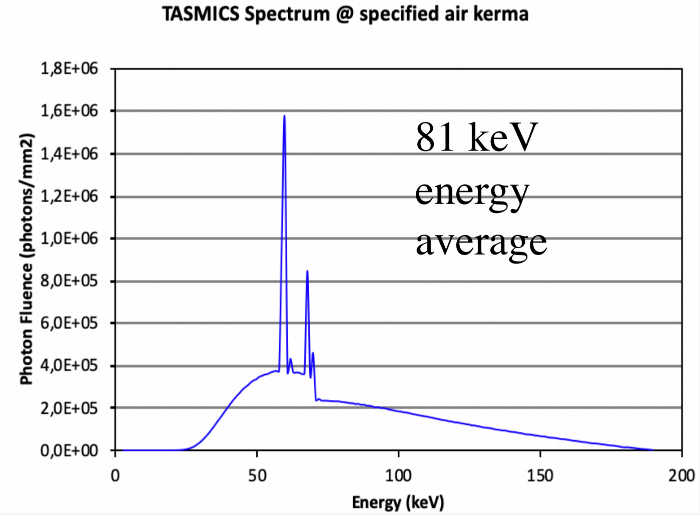
190 kV measurements

MDAMB231
irradiationat **1** and **2** Gy
(nominal dose)

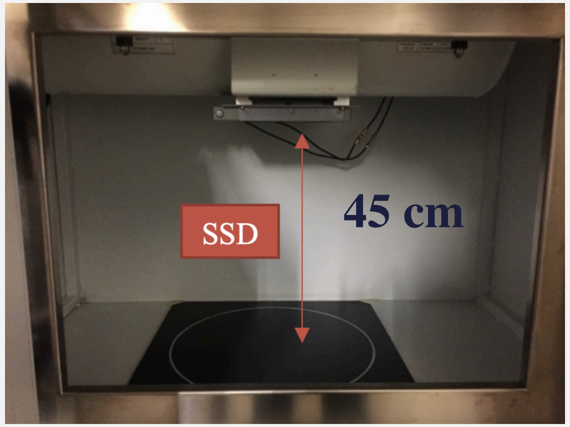
- **0 μg/ml (control)**
- **100 μg/ml**
- **200 μg/ml**

15 nm AuNP





$$SF\ ratio = \left.\frac{SF_{No\ AuNP}}{SF_{AuNP}}\right|_{Gy}$$



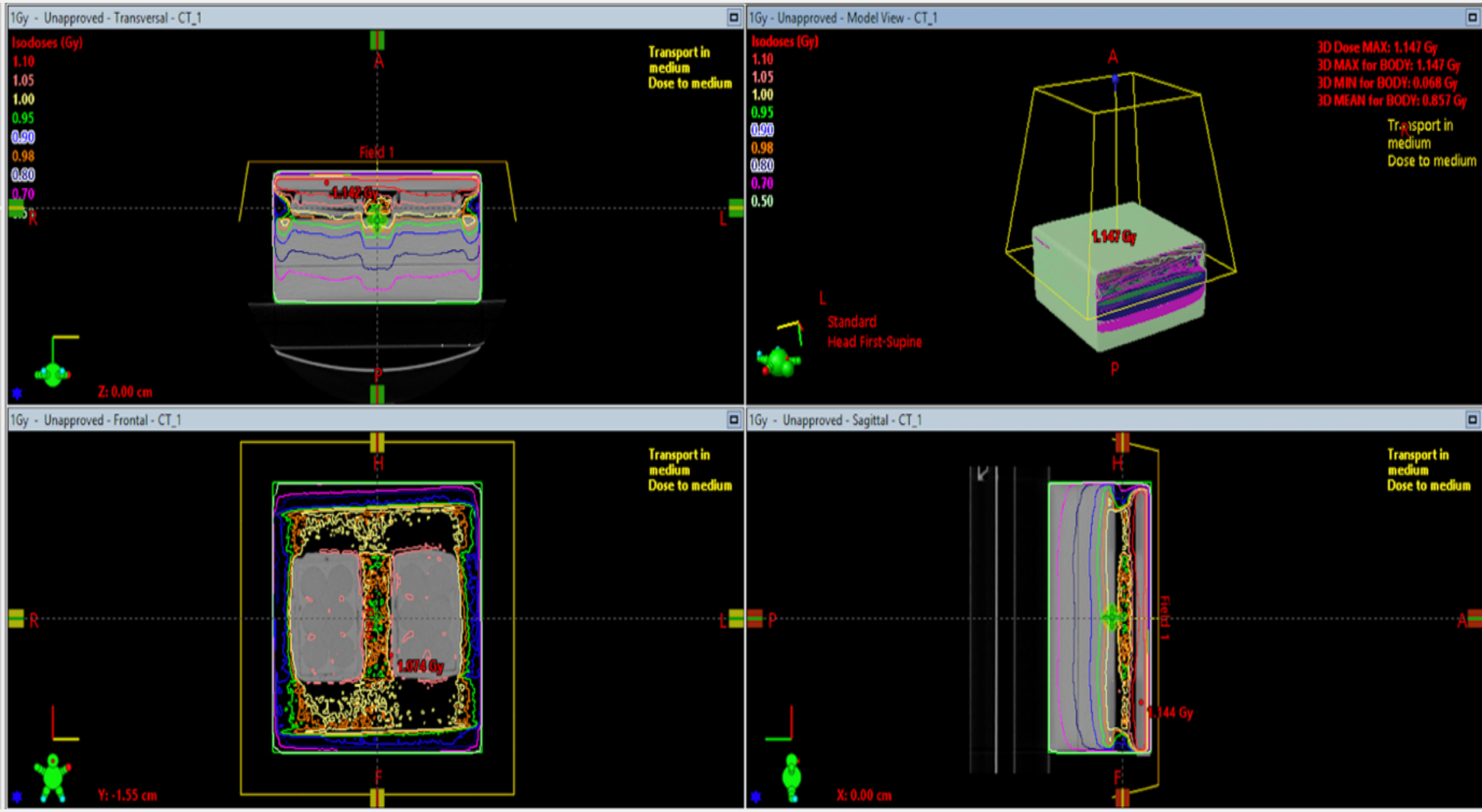
(results of one measurement)

CELL LINE	X-ray tube potential	AuNP CONCENTRATION	AuNPs size	RATIO OF SURVIVAL FRACTIONS AT 1 Gy (nominal dose)	RATIO OF SURVIVAL FRACTIONS AT 2 Gy (nominal dose)
MDAMB231	190 kV	100 μg/ml	15 nm	**2.3±0.3**	**2.1±0.3**
MDAMB231	190 kV	200 μg/ml	15 nm	**3.2±0.3**	**2.9±0.3**

6 MV measurements at San Raffaele Hospital

Varian Clinac iX System Linac

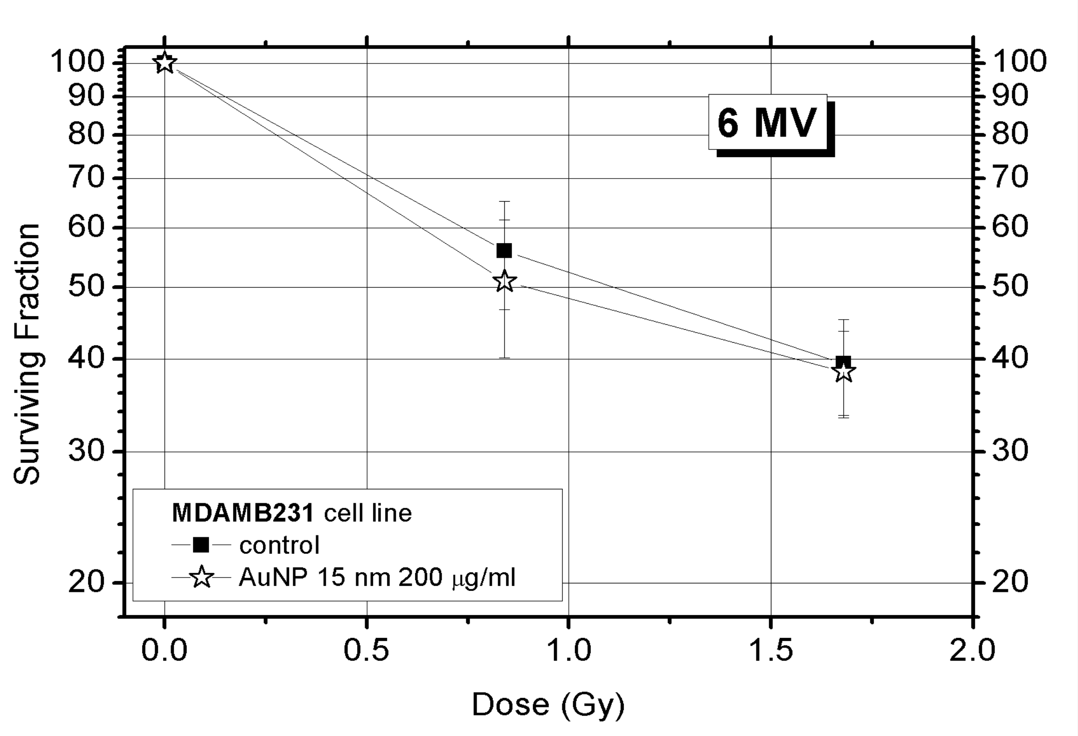
Eclipse ™ treatment planning system



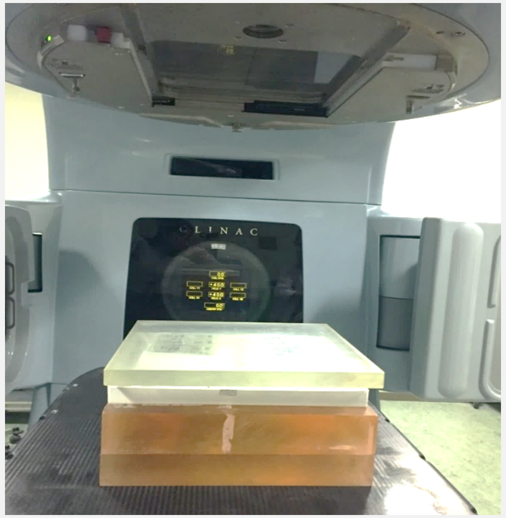
- CT images acquisition
- Contouring target and critical structures areas
- Dose distribution calculation

E. Vanetti et al. *The impact of treatment couch modelling on RapidArc* , Physics in Medicine and Biology , **54**, N157–N166, (2009).
Halperin E et al., *Perez and Brady's principles and practice of radiation oncology (5th ed.)*. Philadelphia: Lippincott Williams and Wilkins. P.152. ISBN 9780781763691

6 MV measurements at San Raffaele Hospital



(results of two measurements)



MDAMB231 and irradiation at **1** and **2** Gy (nominal dose) →

- **0 μg/ml (cntrl)**
- **200 μg/ml of 15 nm AuNP**

CELL LINE	X-ray tube potential	AuNP concentration	AuNPs size	RATIO OF SURVIVAL FRACTIONS AT 1 Gy (nominal dose)	RATIO OF SURVIVAL FRACTIONS AT 2 Gy (nominal dose)
MDAMB231	6 MV	200 μg/ml	15 nm	**1.1±0.3**	**1.0±0.2**

Our results

Cell line	X-ray tube potential	AuNP concentration	AuNP size	DEF at 50% Survival fraction
MDAMB231	190 kV	100 μg/ml	15 nm	2.3 ± 0.3
MDAMB231	190 kV	200 μg/ml	15 nm	2.8 ± 0.3
MDAMB231	6 MV	200 μg/ml	15 nm	1.4 ± 0.4
MDAMB231	100 kV	100 μg/ml	15 nm	1.2± 0.3
MDAMB231	100 kV	200 μg/ml	15 nm	1.3±0.3

$$DEF = \left.\frac{D_{NO\ AuNP}}{D_{AuNP}}\right|_{50\%\ SF}$$

Cell line	X-ray tube potential	AuNP concentration	AuNP size	SURVIVAL FRACTION RATIO at 1 Gy (nominal dose)	SURVIVAL FRACTION RATIO at 2 Gy (nominal dose)
MDAMB231	190 kV	100 μg/ml	15 nm	2.3 ± 0.3	2.1 ± 0.3
MDAMB231	190 kV	200 μg/ml	15 nm	3.2 ± 0.3	2.9 ± 0.3
MDAMB231	6 MV	200 μg/ml	15 nm	1.1 ± 0.3	1.0 ± 0.2
MDAMB231	100 kV	100 μg/ml	15 nm	2.3 ± 0.3	1.0± 0.3
MDAMB231	100 kV	200 μg/ml	15 nm	3.2 ± 0.3	1.3± 0.3

$$SF\ ratio = \left.\frac{SF}{SF_{AuNP}}\right|_{Gy}$$

Medical Physics Research Laboratory

Conclusions

- Gold nanoparticles enhanced radiotherapy is an active field of research, though initiated several years ago

- A group of Italian Medical Physicists, funded by INFN, proposed a new Radiotherapic approach for breast cancer treatment that consist in using of **kilovoltage** energies with the injection of **Au nanoparticles** in tumours, in order to maximize locally radiation effects

- They observed a significant Dose Enhancement effect upon cellular uptake of 15 nm gold nanoparticles at 190 kV, higher than100 kV

- kV photons produce higher Dose Enhancement than MV photons

Grazie per la Vostra gentile attenzione!

Alessia Tudda

a.tudda@na.infn.it