Radiosensitization of Cancer Cells using Nanoparticles in X-ray and Ion Beam Therapy

L. Sihver[1,2], I. Tremi [3, 4], E. Spyratou [3], M. Makropoulou [4], E. P. Efstathopoulos [3], and A. G. Georgakilas [3]

1. Chalmers University of Technology, Gothenburg, Sweden
2. Technische Universität Wien, Atominstitut, Vienna, Austria
3. National and Kapodistrian University of Athens, Athens, Greece
4. National Technical University of Athens, Athens, Greece

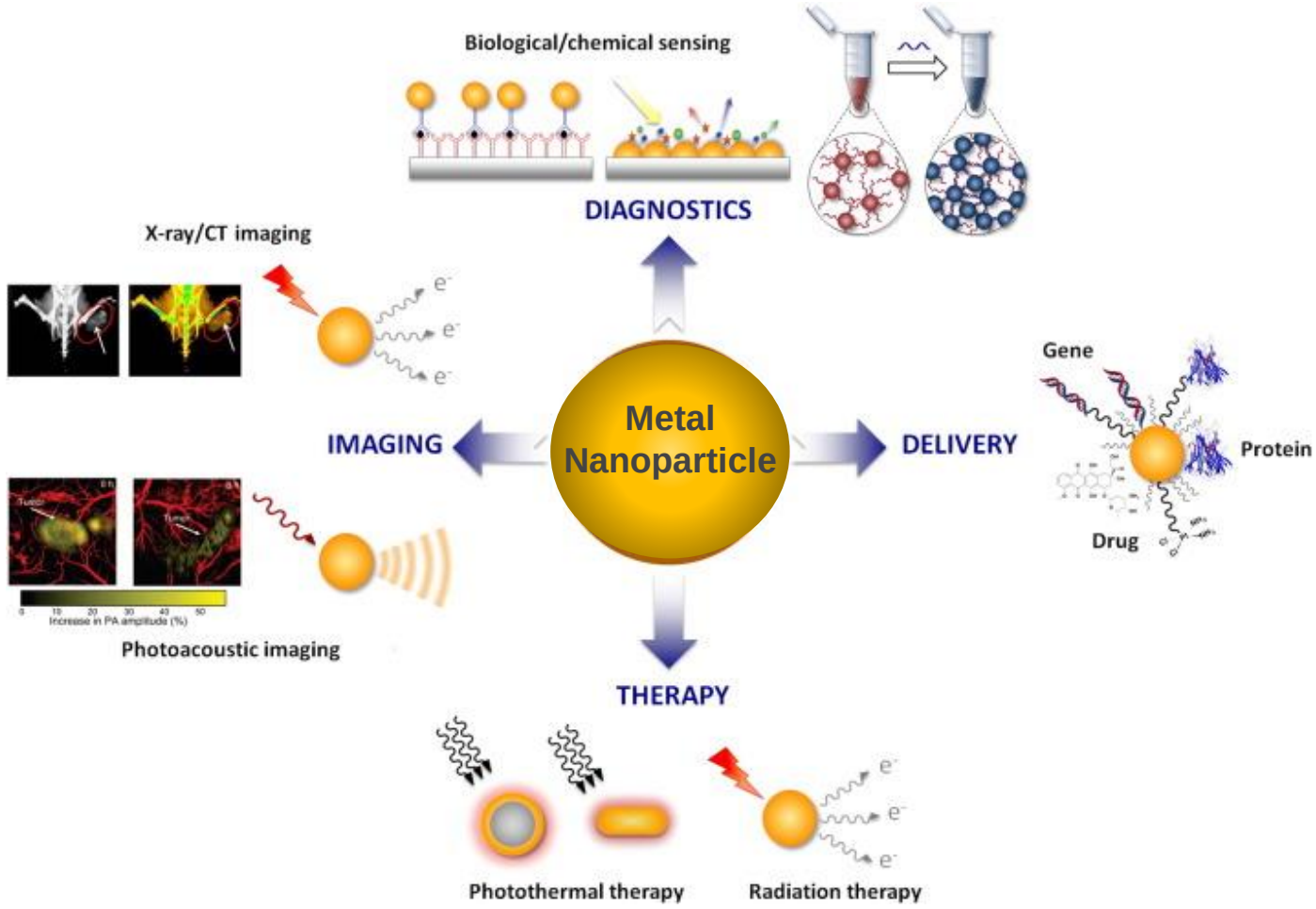
Content

- Improve Effectiveness of Cancer Therapy
- Biomedical Applications of Nanoparticles (NPs)
- Cell Radiosensitization
- Enhancing the Radiosensitization in Cancer Cells
- Nanoparticles in X-ray and Ion Beam Therapy
  - Effects of Size, Shape, and Surface Treatment
  - Biological / Biochemical Effects
- Experimental Procedures to Study the Biological Effects of NPs' Radiosensitization
- Clinical Trials
- Summary and Conclusions

Improve Effectiveness of Cancer Therapy

- Many different tumor-targeted strategies have been developed to limit the side effects and improve the effectiveness of cancer therapies, e.g.:
  - Chemotherapy
  - Intensity modulated radiation therapy
  - Biology-driven personalized radiotherapy
  - Ion beam radiotherapy
  - Target-alpha-therapy
  - High intensity focused ultrasound therapy
- There are also new strategies under development, e.g.:
  - FLASH (ultra-high dose rate radiation therapy)
  - Radiosensitization of cancer cells using metal NPs

Biomedical Applications of NPs



Her et al., 2015, Adv Drug Deliv

Cell Radiosensitization

The Law of Bergonie & Tribondeau

- In 1906, only 11 years after the discovery of ionizing radiation, Bergonie and Tribondeau carried out an experiment in which irradiation to the testis of mice was used to determine the sensitivity of tissue. They found that the sensitivity of cells to radiation is proportional to the degree of proliferative activity and inversely proportional to the degree of differentiation.

→ Undifferentiated cells with high mitotic capability are more radiosensitive.

Bergonie & Tribondeau

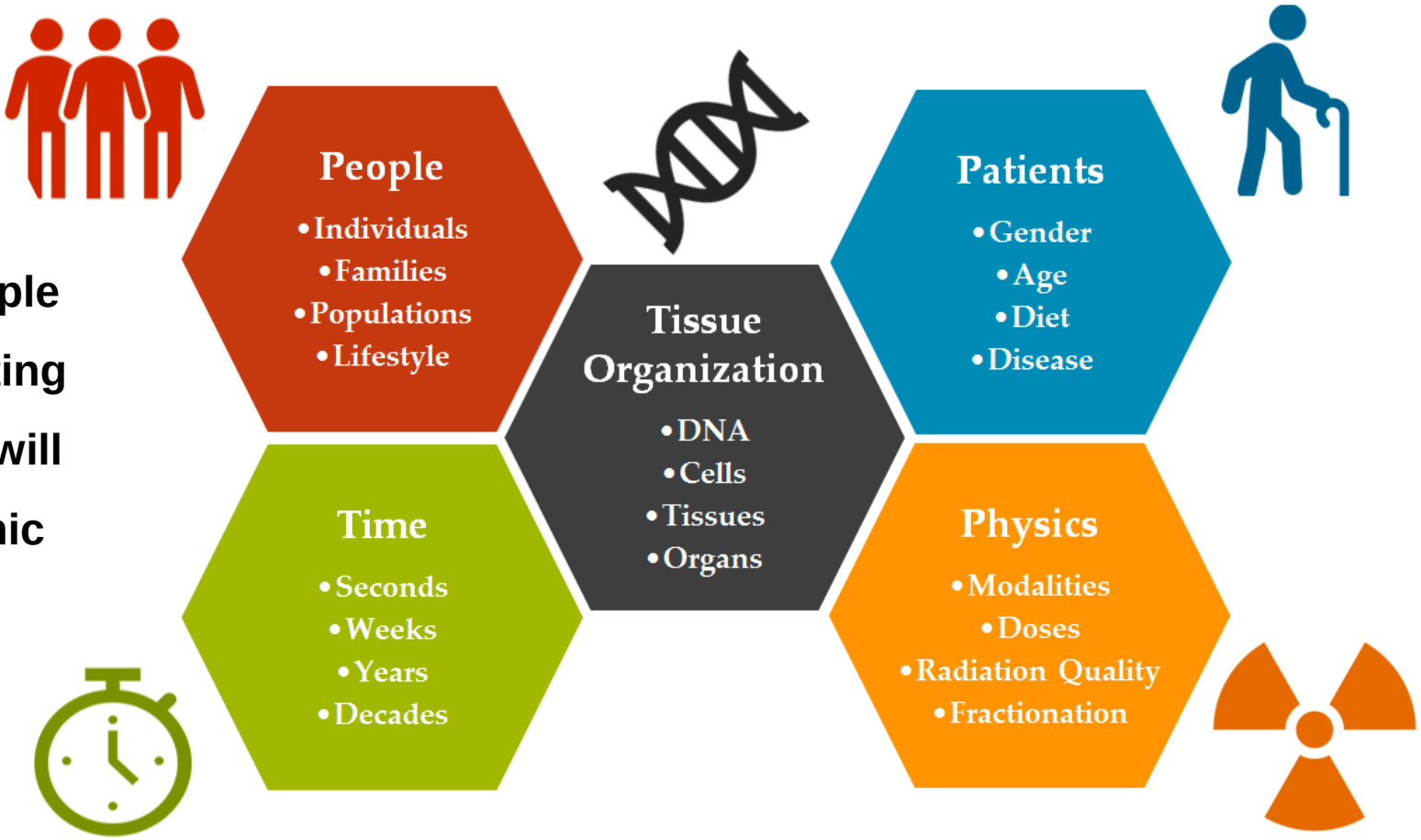
- 1906 French Scientists researching that Stem cells are MORE radiosensitive
- Younger tissues & organs are radiosensitive
- ↑ Metabolic activity, ↑ radiosensitivity
- ↑ proliferation & growth rate, ↑ radiosensitivity

LOMA LINDA UNIVERSITY
School of Allied Health Professions
Medical Radiography Program

From: https://slideplayer.com/slide/13067806/

The Multi-Dimensional Problem of Radiosensitivity

- **Radiosensitivity exists across multiple scales of people and time, contributing to the difficulties in predicting who will be sensitive to toxic and carcinogenic effects of radiotherapy in advance.**

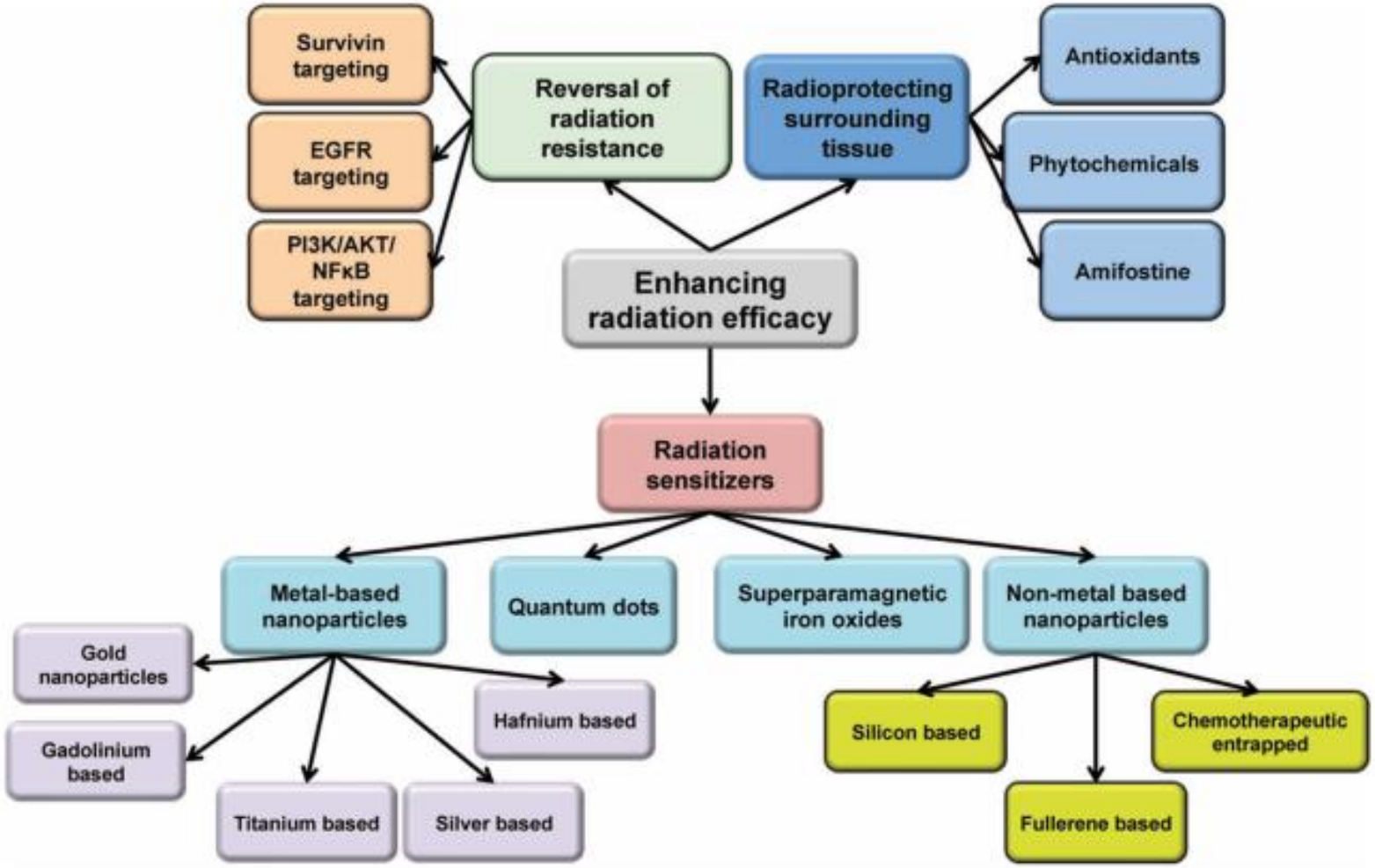


M. Habash, et al., Clinical and Functional Assays of Radiosensitivity and Radiation-Induced Second Cancer. Cancers 2017, 9, 147; doi:10.3390/cancers9110147

Radiosensitivity and Radioresistance

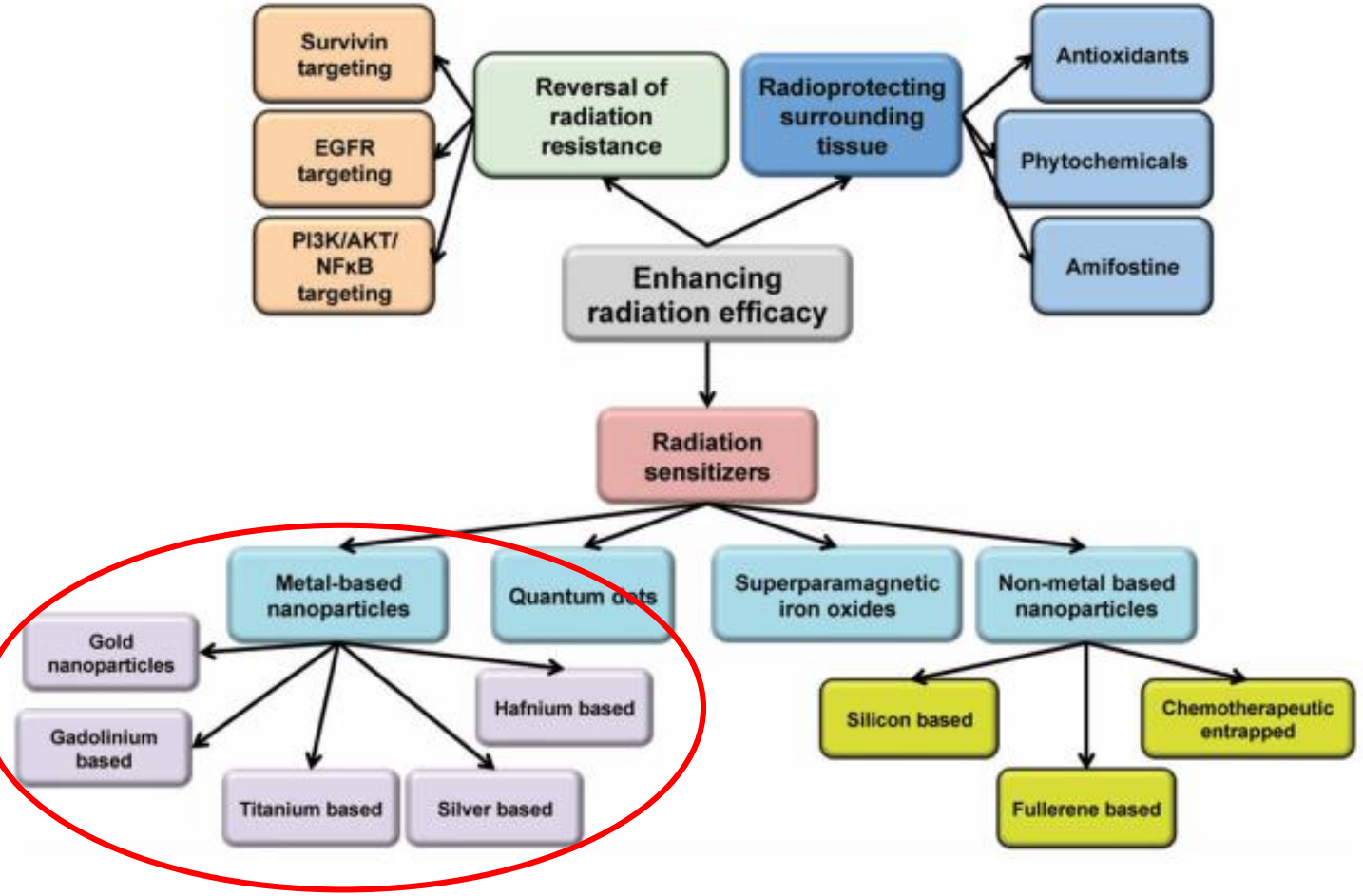
- **In human cancer radiotherapy a major obstacle is the radioresistance of hypoxic neoplastic cells.**
  - **Hypoxic cells develop in tumors because of the unbalanced growth of cancerous cells and of the vascular components needed to provide an adequate blood supply, as was first reported early in 1955 by Thomlinson and Gray.**
- **It is therefore important to find methods to improve the effectiveness of cancer therapies by enhancing the radiosensitization of the cancer cells while reducing or maintaining the normal tissue complication probability during radiation therapy.**

Enhancing the Radiosensitization in Cancer Cells



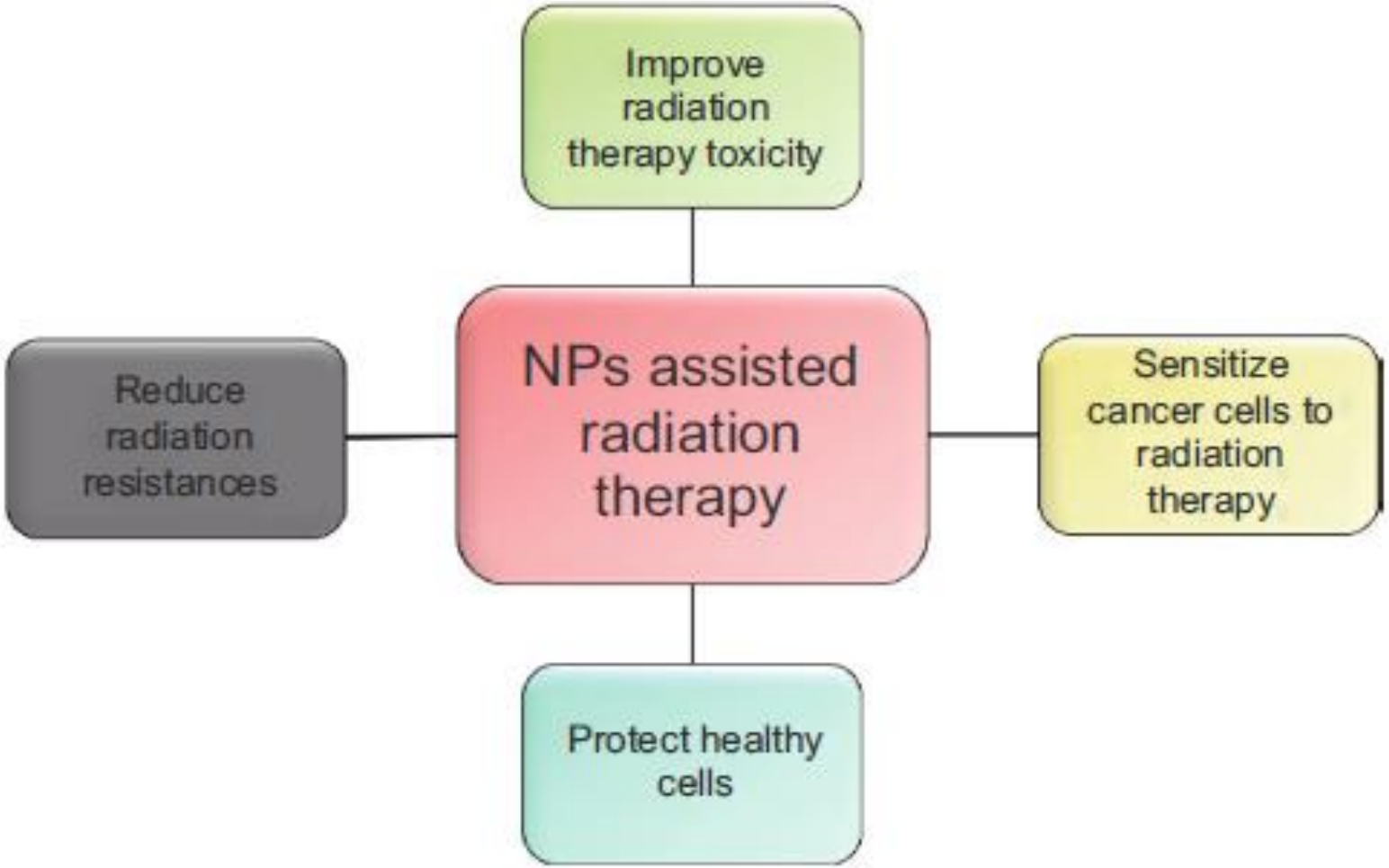
There are many different approaches for enhancing the radiosensitization of cancer cells

Enhancing the radiosensitization in cancer cells



There are many different approaches for enhancing the radiosensitization of cancer cells

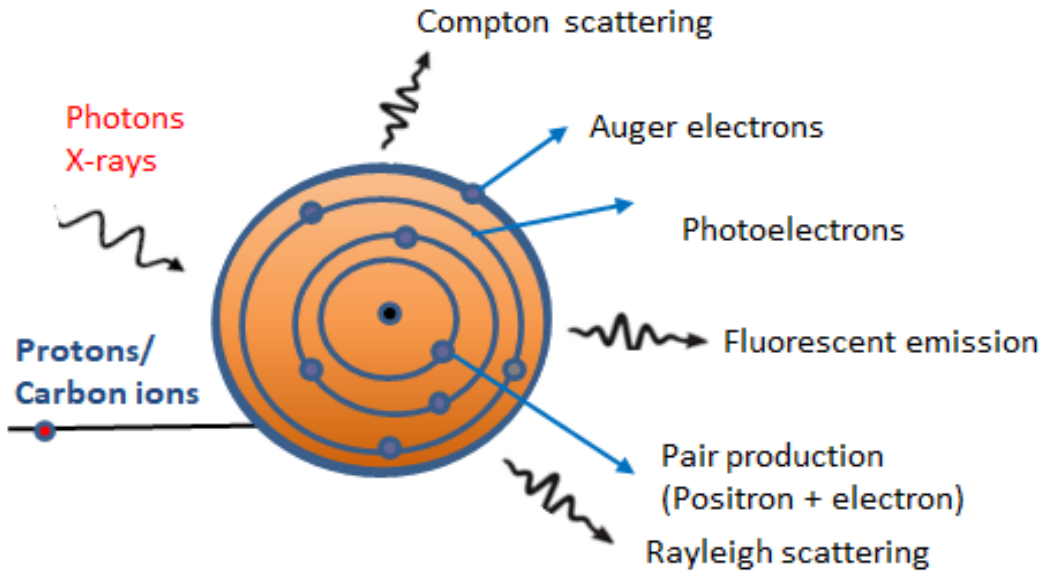
NPs Assisted Radiation Therapy



Schematic view on how NPs can improve the efficacy of radiation therapy

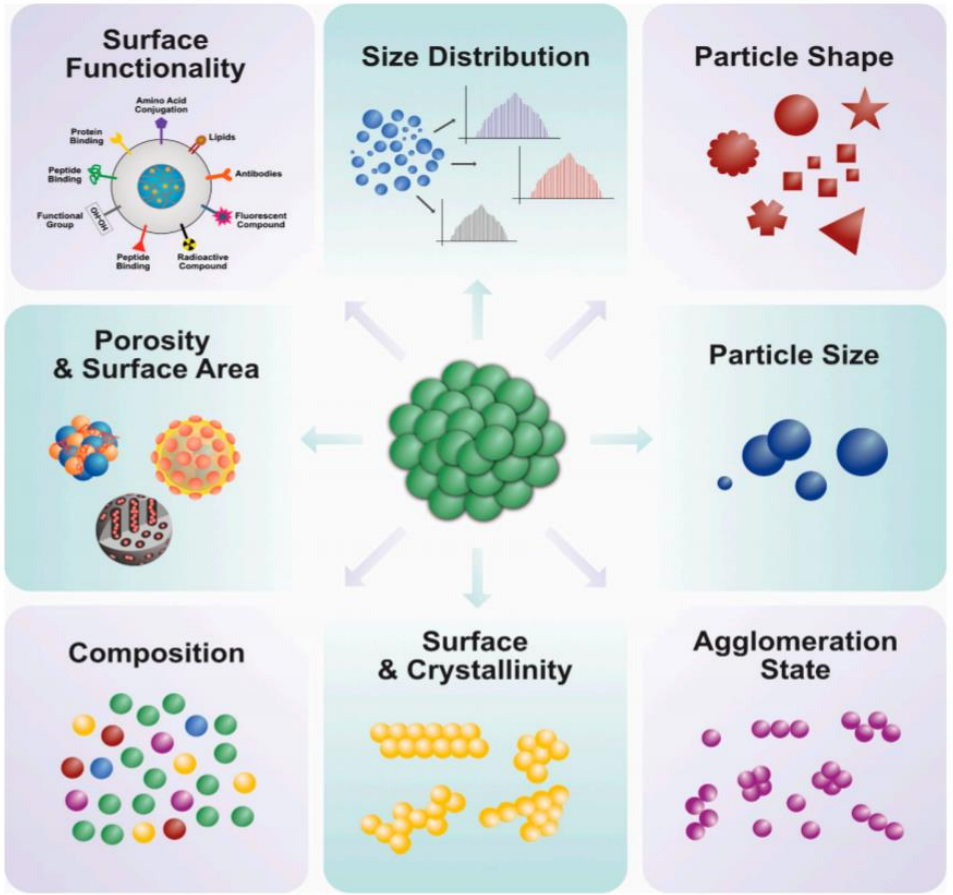
Metal NP Enhancement of Radiosensitization in Cancer Cells

- **When X-rays, protons or a heavier ion hit a metal, there is an amplified production of many tumor destructive components.**
  - **The most relevant to cancer radiotherapy are scattered X-rays/photons, photoelectrons, Compton electrons, Auger electrons and fluorescence photons.**



Metal NPs in X-ray and Ion Beam Therapy

- **Different metal-based NPs have been evaluated to enhance the radiosensitization of the cancer cells during radiation therapy, e.g.:**
  - **Platinum, Gold, Gadolinium, Titanium, Silver, Hafnium, Bismuth, and Iron.**
- **Many different surface chemistries, sizes, compositions, shapes, etc. are possible.**



https://www.mdpi.com/2079-4991/9/12/1719/htm

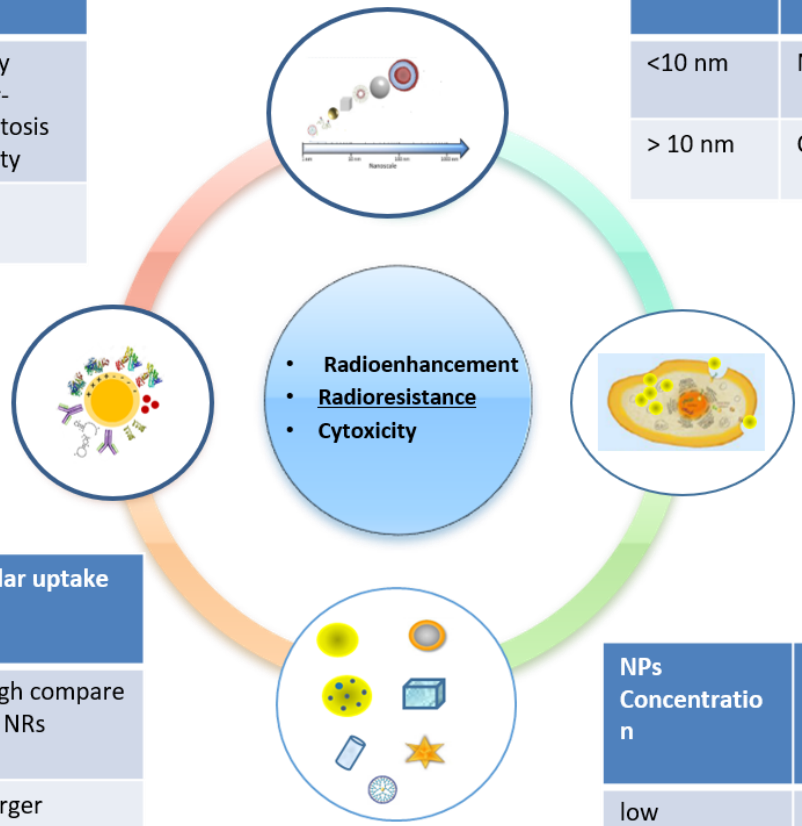
Key Parameters for Designing an Effective NP-based Radiosensitizing Effect in RT

Varity of Sizes

NPs size	Binding ability
~ 10 nm	• Inability to occupy multiple receptor-mediated endocytosis • Low binding avidity
~ 50 nm	• Effective cellular uptake

NPs size	Intracellular location	Circulation time
<10 nm	Nuclear uptake	Easily be cleared via kidneys
> 10 nm	Cytoplasm	Adequate residence time

Different Surface Functionalization



Different Concentration and Intercelluar Locations Functionalization

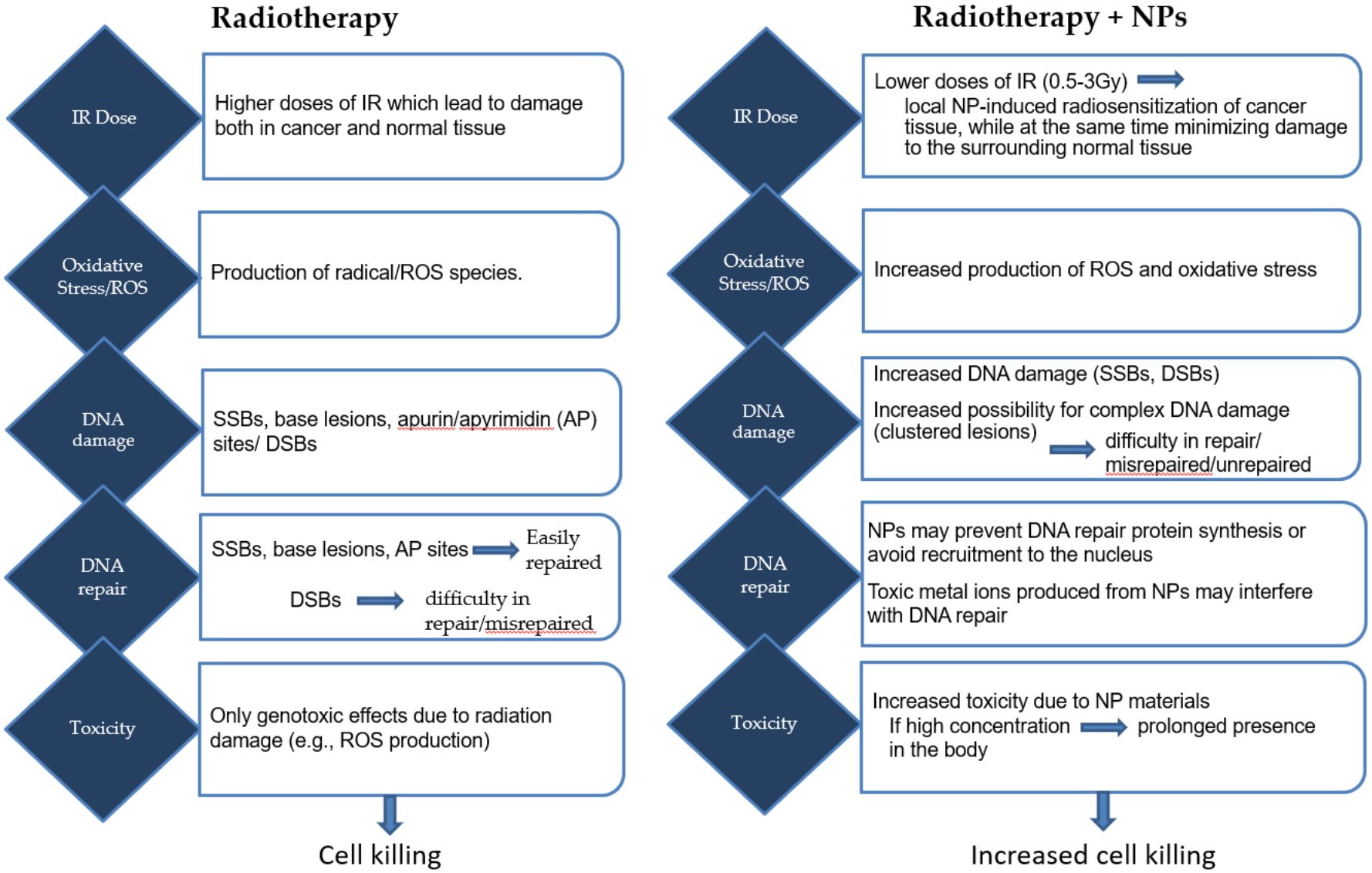
NPs shape	Surface functionalization	Cellular uptake
Nanospheres (NSs)	• Cationic • PEG	• High compare to NRs
Nanorods (NRs)	• None	• Larger contact area with cell membrane • better endocytosed compared to NSs

Varity of Shapes

NPs Concentration	Distribution	Cytoxicity
low	• low	• low
High	• High • increase probability of aggregation	• Higher degree of toxicity

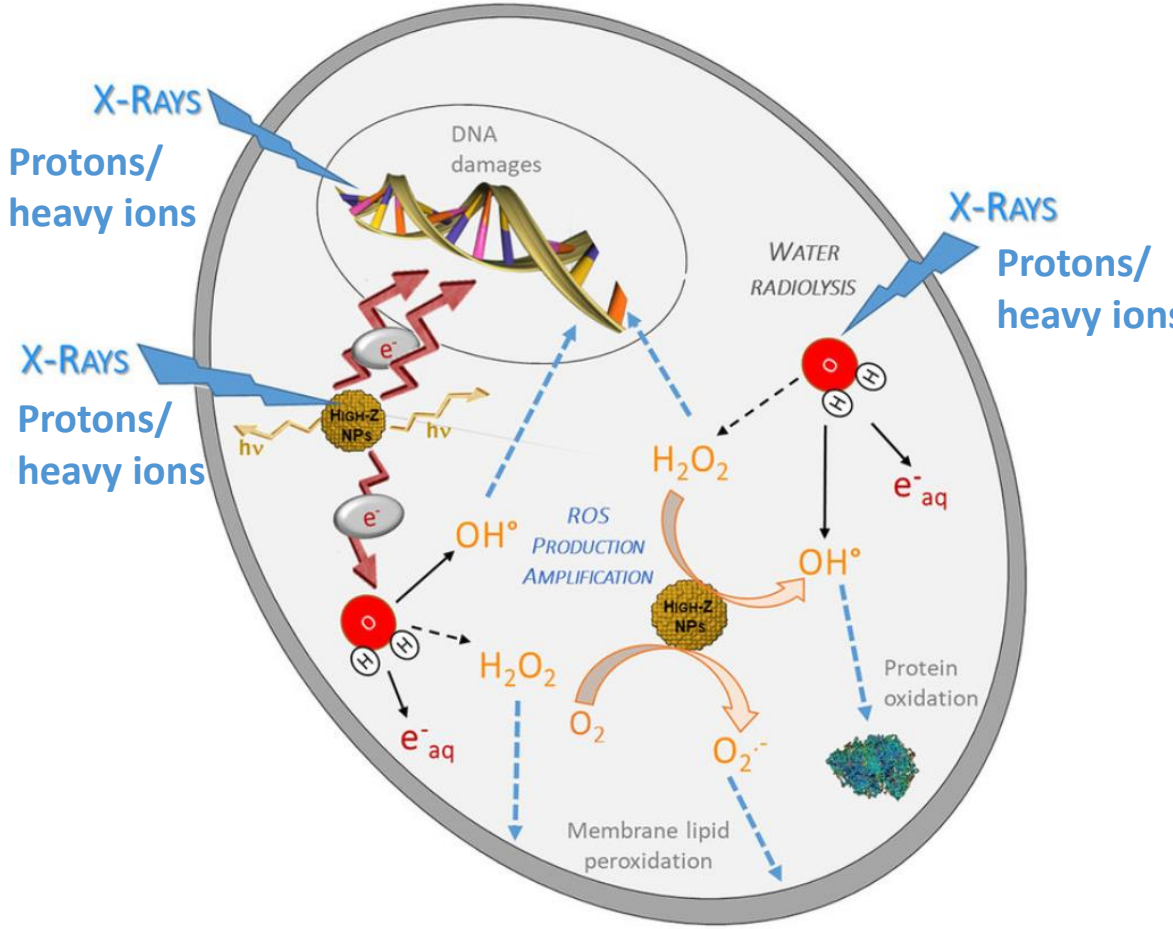
Prof. Dr. L. Sihver

Metal NP Enhancement of Radiosensitization in Cancer Cells



Metal NPs in X-ray and Ion Beam Therapy

- **Incident X-ray/photon beam induce excitations and ionizations of intracellular components in the cells, causing:**
  - **Direct effects such as DNA damages (e.g. DNA single and double-strand breaks, DNA-protein crosslinks).**
  - **Water radiolysis leading to radical and reactive oxygen species (ROS) production, which leads to indirect effects.**
- **The direct and indirect effects leads to biological effects.**

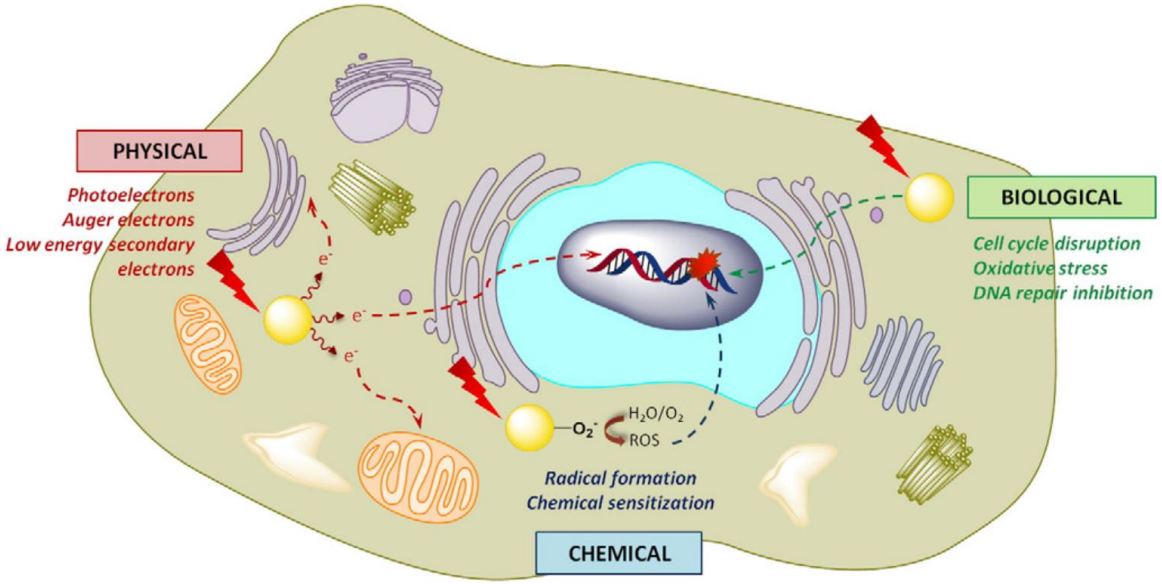


Schematic illustration of radiotherapy enhancement by metal-based nanoparticles.

Metal NPs in Radiotherapy as Radiosensitizers

3 Mechanisms Leading to Dose Enhancement

- **Physical mechanisms -> Direct effects**
  - Compton scattering
  - Photoelectric effect
  - Auger electron emission
- **Chemical mechanisms -> Indirect effects**
  - Low energy electrons (LEEs)
    - ✓ Increase radio sensitization by weakening the bonds of DNA
    - ✓ Creating reactive oxygen species (ROS)
    - ✓ Creating radicals
- **Biological mechanisms -> Biological effects**
  - Increase oxidative stress
  - Cell cycle disruption
  - DNA repair inhibition
  - Alter cellular macromolecules inducing protein oxidation, membrane lipid peroxidation



Advanced Drug Delivery Reviews 109 (2017) 84–101

Metal NPs in X-ray and Ion Beam Therapy

- **When using X-rays, the energy is important, since the photoelectric effect is decided by $(Z/E)^3$, where:**
  - **E = the energy of the incoming photon**
  - **Z = the atomic number of the nucleus being targeted**
  - **The photoelectric effect is dominant at lower energies and is prevailing until the photon energy reaches a medium energy (e.g., around 500 keV for gold (Z = 79)) with a cross section varying with Z^4 or Z^5, depending on the material.**
  - **When using X-rays, mainly the inner electron shells are ionized, which creates cascades of both low and high energy Auger electrons.**

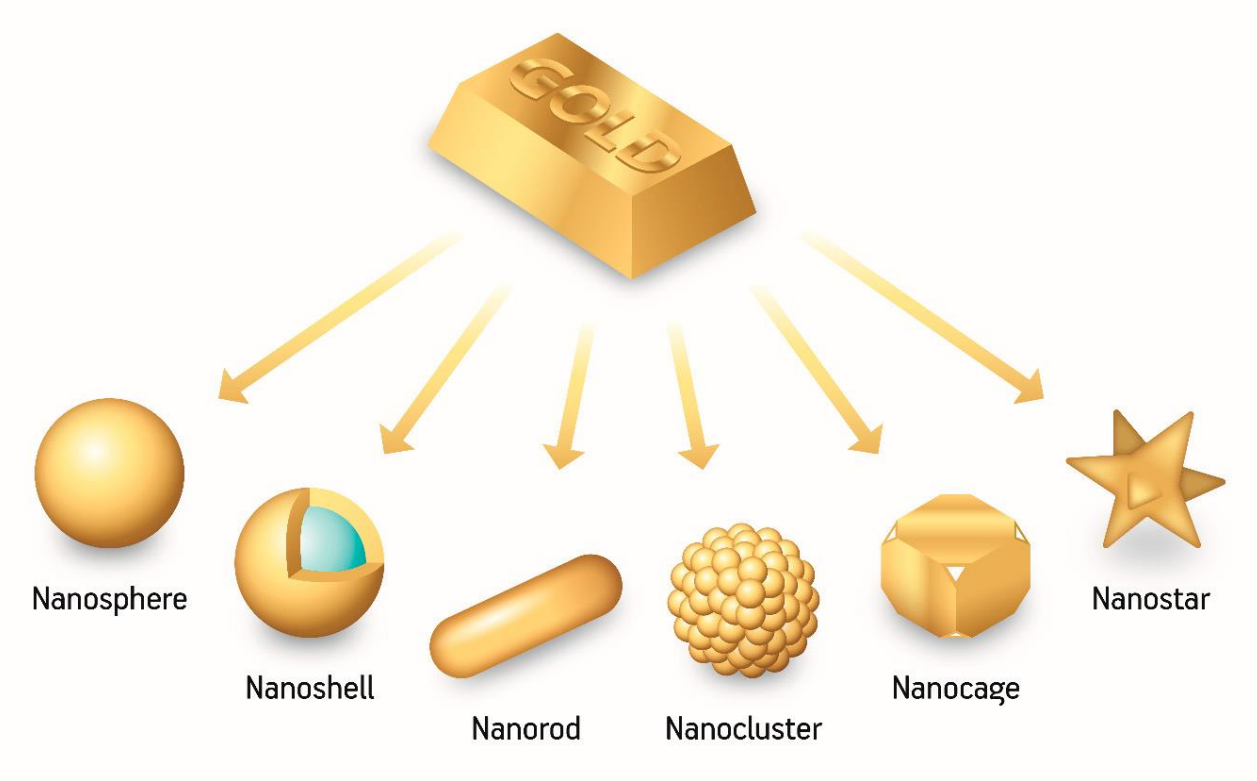
Metal NPs in X-ray Therapy

- **The use of higher Z metal NPs (e.g., gold or platinum) along with X-rays, leads to enhanced photoelectric and Compton effects.**
  - **This result in an amplified production of secondary electrons and ROS, contributing to enhanced cytotoxic effects on irradiated cells, making these NPs more radiosensitizing than others with lower Z.**
- **They can also be used as imaging contrast agents, to be used in disease diagnosis as well as for biological imaging.**

Gold (Au) NPs in X-ray Therapy

- **Gold NPs (AuNPs) are promising radiosensitizers because of:**
  - **High Z ➡ higher mass energy absorption coefficient in relation to soft tissue.**
  - **Very inert, highly biocompatible and has low biological toxicity when compared with traditional agents.**
  - **High surface area to volume ratio that allows drugs and other therapeutic agents (e.g., peptides, proteins, antibodies, small molecules) to attach to their surface for targeted treatment and combination therapy of tumors.**
  - **Can enter the cell interior through energy independent processes such as simple diffusion, or through the energy-dependent process, endocytosis.**
  - **Well-controlled size distribution.**
  - **Unique chemical, electrical, and optical properties in the range of 1–150 nm.**

Gold NPs in X-ray and Ion Beam Therapy

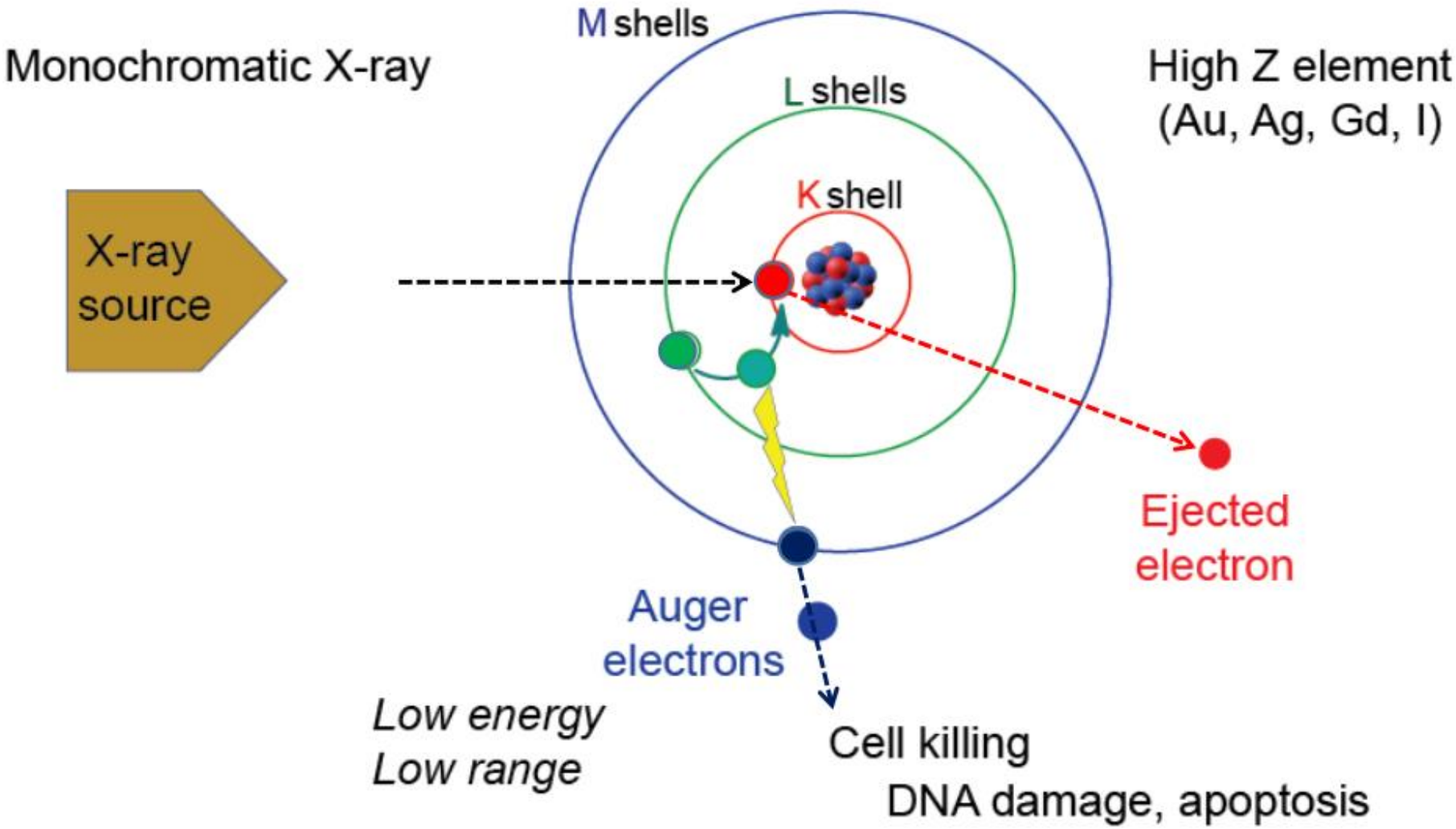


Most common AuNP assemblies and morphologies.

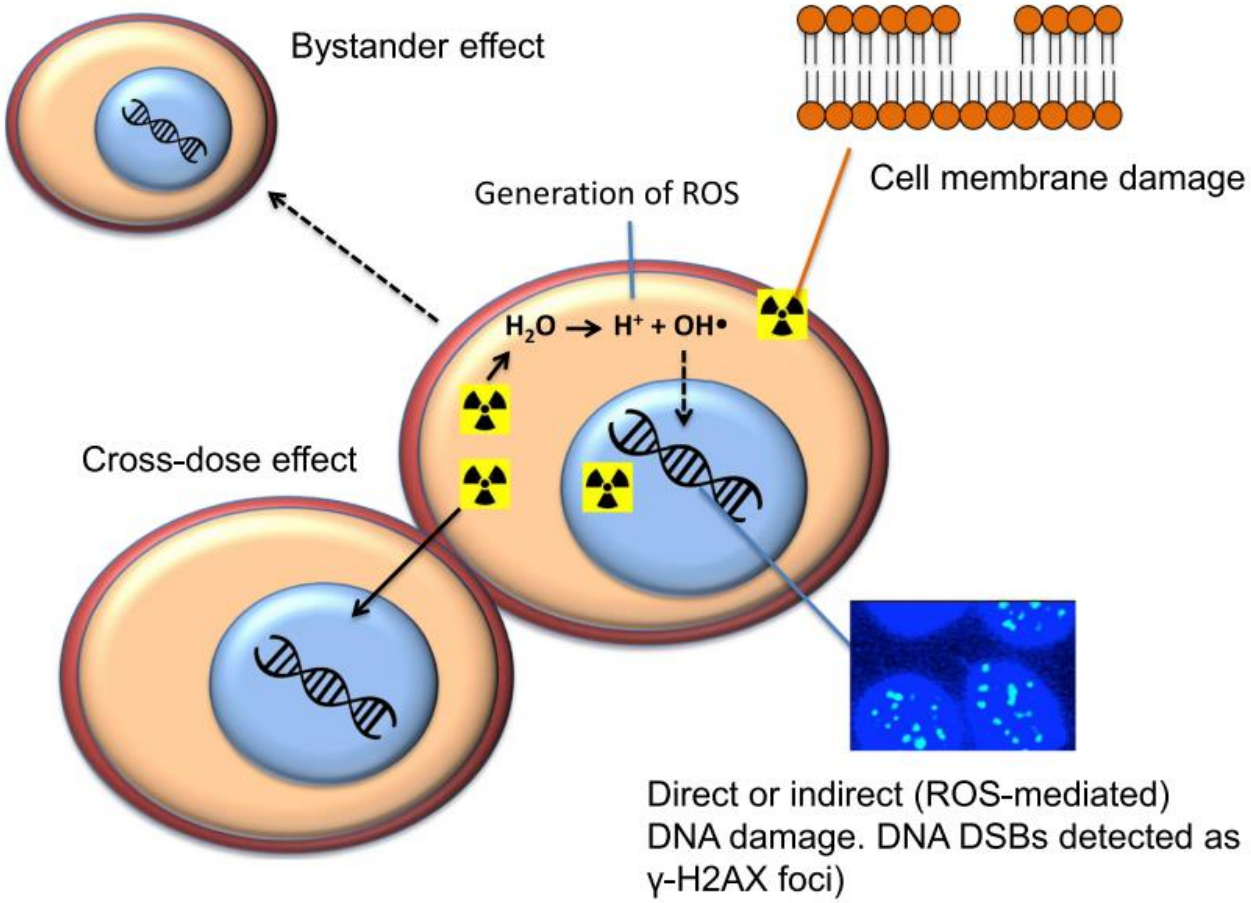
Irradiation of High Z Metal NPs with Monocromatic X-rays

- **Irradiation of high Z element such as gold, silver or gadolinium with a monochromatic X-ray causes photoelectric effect involving inner shell ionization.**
- **If the monochromatic X-ray has an energy same or higher than the K-shell edge energy of the metal, an electron in the K-shell will be kicked out of the atom.**
  - **This results in the movement of an electron from outer shell to the K-shell.**
  - **This releases energy which is then used to kick out other electrons from the atom.**
- **Thus, the effect of monochromatic X-rays can be amplified by the use of high Z elements, raising the possibility that an Auger effect-based cancer therapy can be developed.**

Irradiation of High Z Metal NPs with Monocromatic X-rays



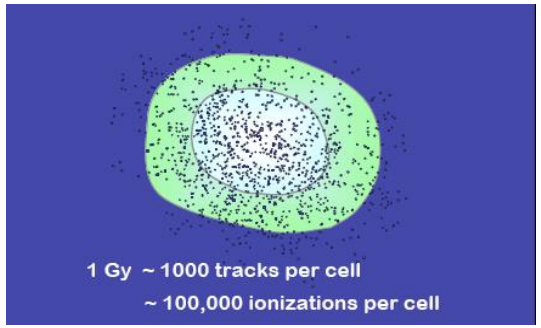
Modes of Cell Death Caused by Auger Electron Emission



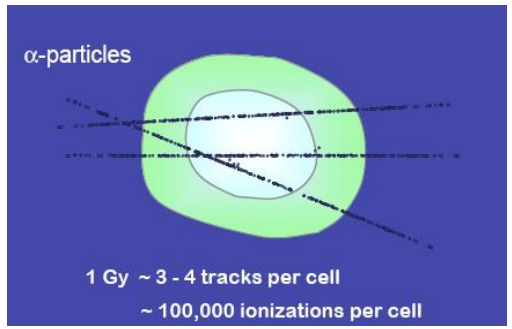
Ku et al. EJNMMI Radiopharmacy and Chemistry (2019) 4:27

Irradiation of High Z Metal NPs with High LET Particles

- **When using high LET particles, e.g., carbon ($^{12}_{6}C$) ions and NPs for therapy:**
  - **Mainly the outer shells are ionized, which produce electrons with lower energies compared to X-rays**
  - **However, the amount of the produced low energy electrons is higher when exposing NPs to ions than when exposing them to X-rays.**
  - **High energetic ions traverse the material along tracks, and therefor give rise to much more inhomogeneous dose distributions than X-rays. There might therefor be a need to introduce a higher amount of NPs when using ions compared to when using X-rays to create enough primary and secondary electrons to get the desired dose escalations.**



Photon radiation deposits energy in a uniform pattern



Particle radiation deposits energy in a non-uniform pattern

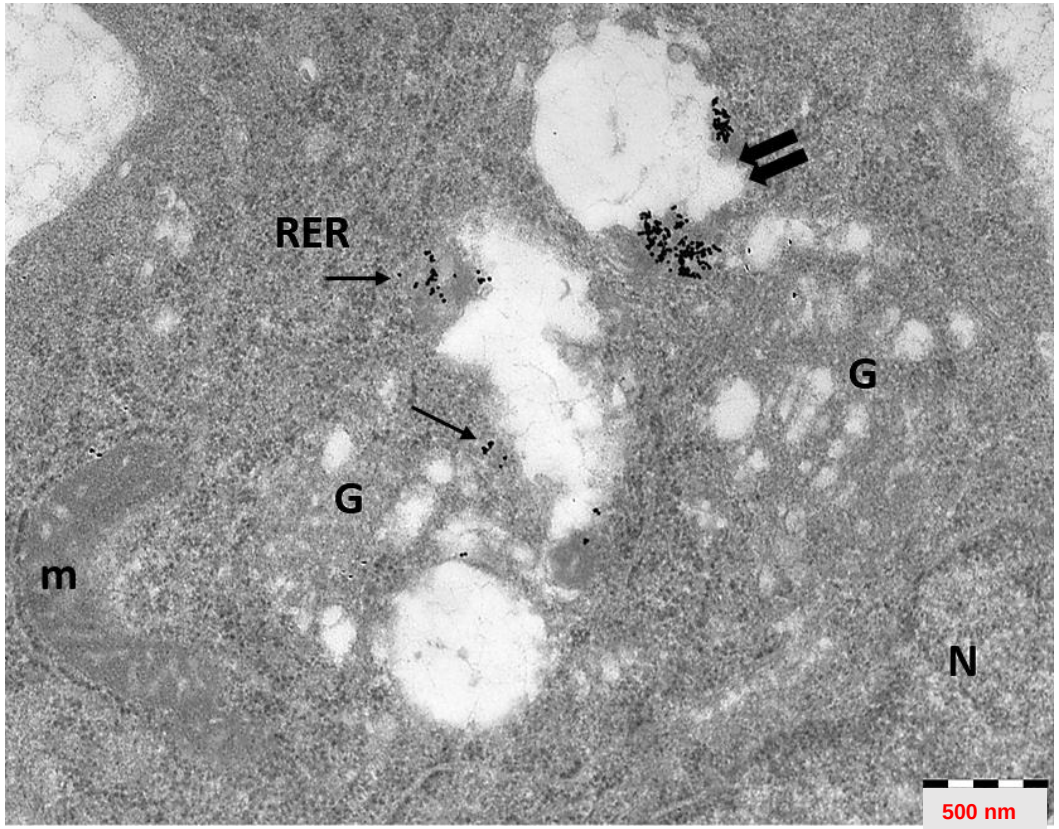
Experimental Procedures to Study the Biological Effects of NPs' Radiosensitization

- Many methods are used to determine the radiosensitization effects of NPs after irradiation.
- A number of different experiments is needed to get a holistic approach and to extract all the possible outcomes of radiosensitization based on DNA damage, oxidative stress, cell survival (e.g., apoptosis, autophagy), cellular senescence, and signaling, e.g.:

Transmission Electron Microscopy (TEM)

- TEM can be used to monitor the cellular uptake of metallic NPs, which play key a role in their radiosensitization effect.
- TEM can be used along with other exp. procedures for identifying and quantifying DNA damage markers after irradiation, in the presence and absence of NPs, for example single or double immunolocalization/immunogold labelling.

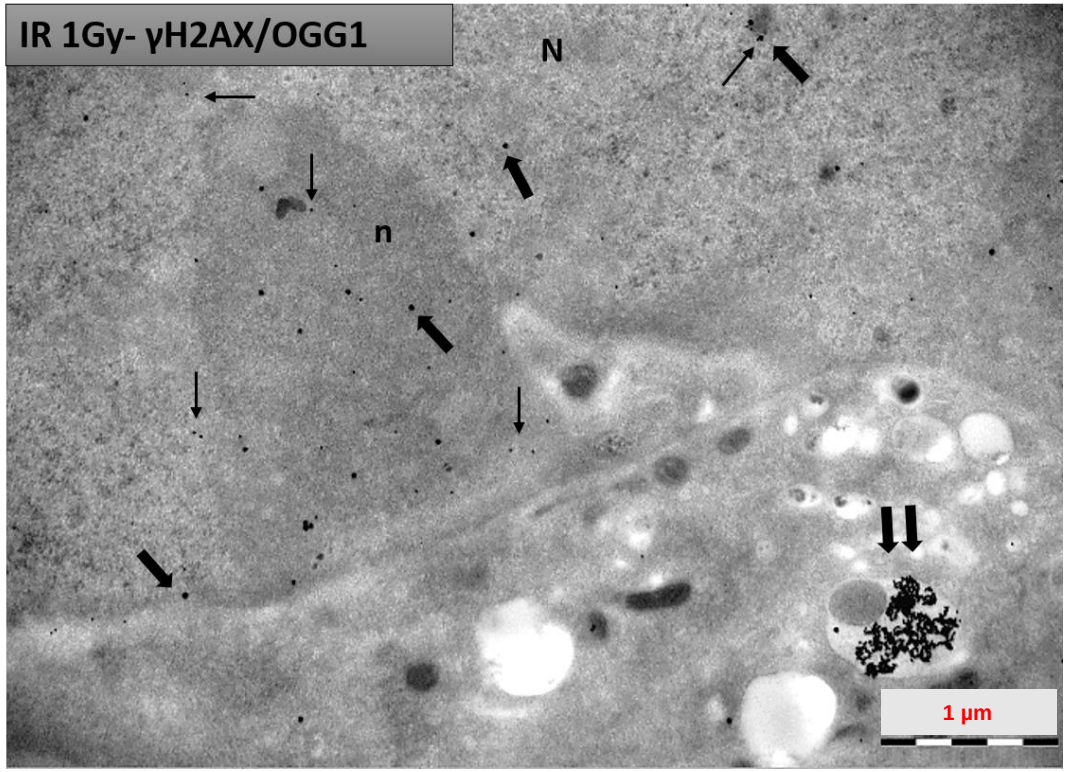
The use of TEM to Study AuNPs Radiosenistation of Prostate Cancer Cells



- **Cellular uptake of 15 nm polyethylene glycol (PEG) capped AuNPs in PC3 (prostate cancer) cells.**
- **Double thick arrows indicate NPs located in vesicles (a structure within or outside a cell consisting of liquid or cytoplasm enclosed by a lipid bilayer) and single thin arrows indicate NPs located in the cytoplasm (a gel like substance between the nucleus and the cell membrane).**
- **N: nucleus, G: Golgi apparatus**
- **RER: rough endoplasmic reticulum**
- **m: mitochondria**

I. Tremi et al. A Guide for Using Transmission Electron Microscopy for Studying the Radiosensitizing Effects of Gold Nanoparticles In Vitro. Nanomaterials 2021, 11, 859. https://doi.org/10.3390/nano11040859

The use of TEM to Study AuNPs Radiosenistation of Prostate Cancer Cells



- PC3 cells after immunogold labelling for DNA damage detection.
- Double thick arrows indicate AuNPs uptake.
- 10 nm immunogold particles and 25 nm immunogold particles are used for labelling γH2AX, which is a marker of DNA double-strand breaks (single thin arrows) and 8-Oxoguanine glycosylase (OGG1), which is involved in DNA base excision repair (single thick arrows), respectively.
- n: nucleolus (the largest structure in the nucleus of eukaryotic cells).

I. Tremi et al. A Guide for Using Transmission Electron Microscopy for Studying the Radiosensitizing Effects of Gold Nanoparticles In Vitro. Nanomaterials 2021, 11, 859. https://doi.org/10.3390/nano11040859

Experimental Procedures to Study the Biological Effects of NPs' Radiosensitization

Flow Cytometry (FC)

- Flow cytometry can be used for DNA damage detection and cell cycle analysis, via Propidium iodide as the most used dye.

Immunofluorescence

- Immunofluorescence uses primary antibodies, labelled with fluorescent secondary antibodies for visualization, specific to targeted DNA repair enzymes. The most common target for DNA damage detection, such as DSBs, is the phosphorylated histone -H2AX (phosphorylation at serine 139).

Agarose Gel Electrophoresis (AGE)

- DNA lesions can be identified through gel electrophoresis. This is a fast method that quantifies the average density of breaks and a variety of DNA lesions in nanogram quantities.

Pulsed-field Gel Electrophoresis (PFGE) and Comet Assay (CA)

- Methods such as PFGE and CA are based on the detection of DNA fragments by electrophoresis.

Experimental Procedures to Study the Biological Effects of NPs' Radiosensitization

Clonogenic Survival Assay (CSA)

- One gold standard technique to access cell death and survival rate in radiobiology.

TUNEL Assay

- TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) staining, also called the TUNEL Assay, detects the DNA breaks formed when DNA fragmentation occurs in the last phase of apoptosis.

Immunoblotting/Western blotting

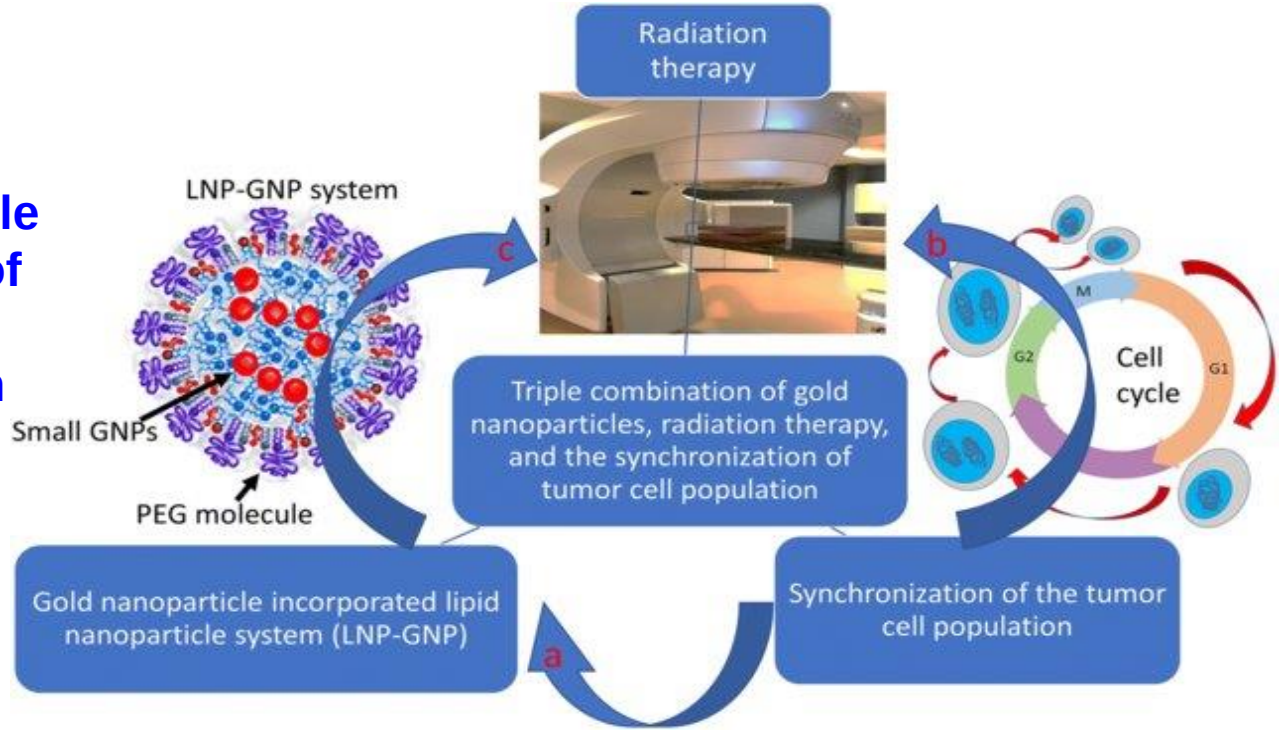
- Immunoblotting, or western blotting, is used to identify changes in protein expression following treatment.

Immunocyto/histochemistry for Cellular Senescence detection

- There is a number of senescence biomarkers.

In Vitro Study of Using NP in RT

Recently, the use of a lipid nanoparticle system as a Trojan horse in delivery of AuNPs to human breast cancer cells was reported, to improve outcomes in radiation therapy.



- **Triple combination of AuNP, radiation therapy and synchronization of the tumor cell population for improved outcomes in cancer therapy.**
- **Synchronization (double thymidine blocking and serum starvation) of the tumor cell population in the right phase could enhance the AuNPs uptake (a) and sensitivity to radiation (b), while having AuNPs within tumor cell can enhance the physical radiation dose (c)**

Bromma et al. Cancer Nano (2019) 10:1, https://doi.org/10.1186/s12645-019-0046-z

Combined NP Radiation Therapies in Completed or Ongoing Clinical Trials.

Name (Sponsor)	Particle Type/Drug	Type of IR/Dose	Name (Sponsor)	Particle Type/Drug	Type of IR/Dose
AGuIX (University Hospital, Grenoble)	Gadolinium-based nanoparticles	X-rays/30Gy (3Gy/session)	NBTXR3 PEP503 (Nanobiotix)	Hafnium oxide- based nanoparticles/PD-I inhibitor	Radiotherapy/-
AGuIX (University Hospital, Grenoble)	Gadolinium-based nanoparticles	X-rays/-	NBTXR3 PEP503 (Nanobiotix)	Hafnium oxide-based nanoparticles	Stereotactic Body Radiation Therapy/45Gy (15Gy/fraction) or 50Gy (5Gy/fraction) External Beam Radiation Therapy/45Gy (1.8Gy/fraction) or Brachytherapy Boost/15Gy + EBRT 45Gy (1.8/fraction)
AGulX (Centre Francois Baclesse)	Polysiloxane and Gadolinium-based nanoparticles	Photon therapy/-	NBTXR3 PEP503 (Nanobiotix)	Hafnium oxide-based nanoparticles	
NBTXR3 PEP503 (Nanobiotix)	Hafnium oxide-based nanoparticles	External beam radiotherapy/ 50Gy(2Gy/fraction)	SPION	Iron oxide Nanoparticles/ ferumoxytol	Stereotactic Body Radiotherapy (LINAC)/-
NBTXR3 PEP503 (Nanobiotix)	Hafnium oxide-based nanoparticles	Intensity-Modulated Radiation Therapy /70Gy (2Gy/fraction)			

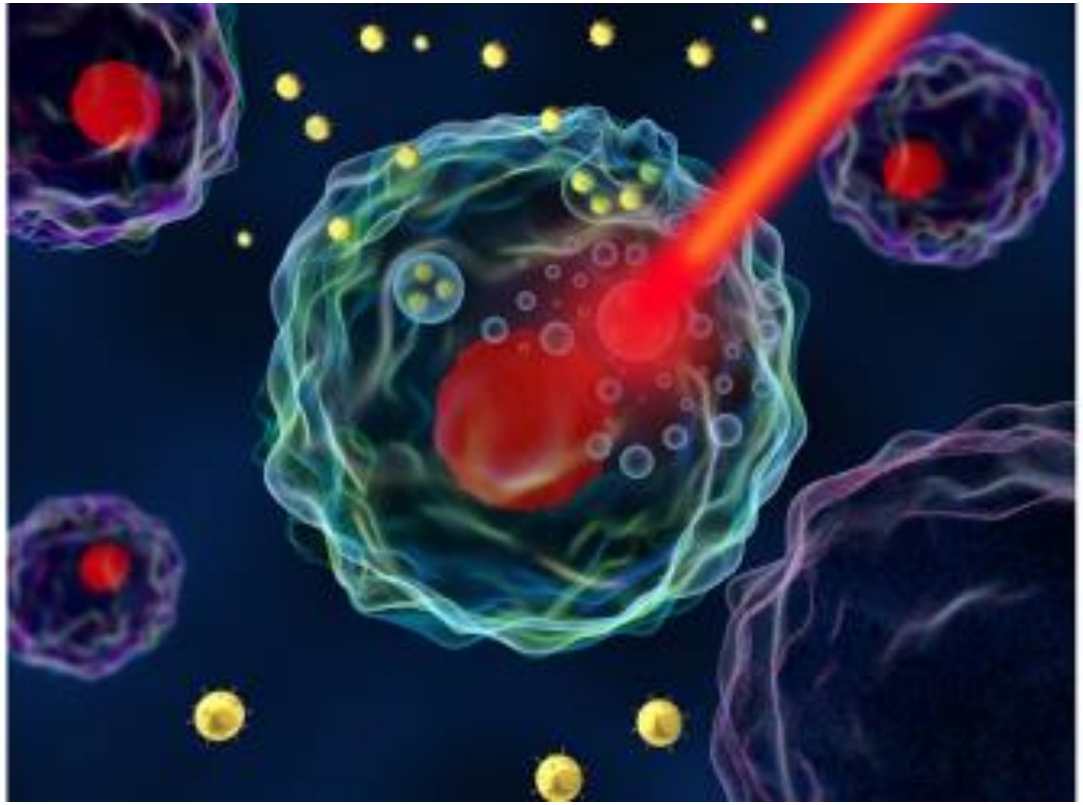
Most trials have been made with X-rays/Photons and Gd, Hf and Au based NPs.

Summary and Conclusions

Mechanisms Leading to Dose Enhancement when using NPs in RT

Physical Effect

- Enhanced photoelectric effect
- Increased photoelectrons
- Increased Auger electrons
- Dominant in KeV than MeV



Chemical/Biological Effect

- ROS production
- Cell cycle effect
- DNA damage
- Enhanced bystander effect

https://jnanobiotechnology.biomedcentral.com/articles/10.1186/s12951-020-00684-5

Summary and Conclusions

Radiosensitization of cancer cells and the impact of NPs in RT

- **The type and energy of ionizing radiation influence the outcome.**
- **The physicochemical properties (size, shape, coating, functionalization, etc.) of nanoagents influence their pharmacokinetics, bioavailability, biodistribution, as well as targeting and intracellular delivery.**
- **The physical and chemical properties of materials are different in the nanometer scale than in macroscale and the size of the NPs used for radiosensitization affects both how they interact with the biological system and how they interact with the incoming radiation.**
- **The sizes of the NPs are a critical parameter.**
  - **Small NPs with sizes ~10 nm are able for nuclear uptake, but they are easily cleared from circulation through kidneys.**
  - **Too large NPs ≥ 100 nm can limit the membrane wrapping efficiency.**

Summary and Conclusions

Radiosensitization of cancer cells and the impact of NPs in RT

- **The concentration of the NPs in combination with the sizes can affect the NPs efficiency and toxicity.**
- **High concentrations of some metallic NPs can increase the probability for aggregations and the toxicity, while NPs can be non toxic at low concentrations.**
- **The surface functionalization of NPs is strongly correlated with the cellular uptake and the subcellular location.**
- **The functionalization can be achieved by coating NPs surface with polyethylene glycol (PEG) or by attaching on their surface antibodies, phospholipids, polymers and other biomolecular linkers depending on chemical moiety that is over expressed in each type of cancer cell.**

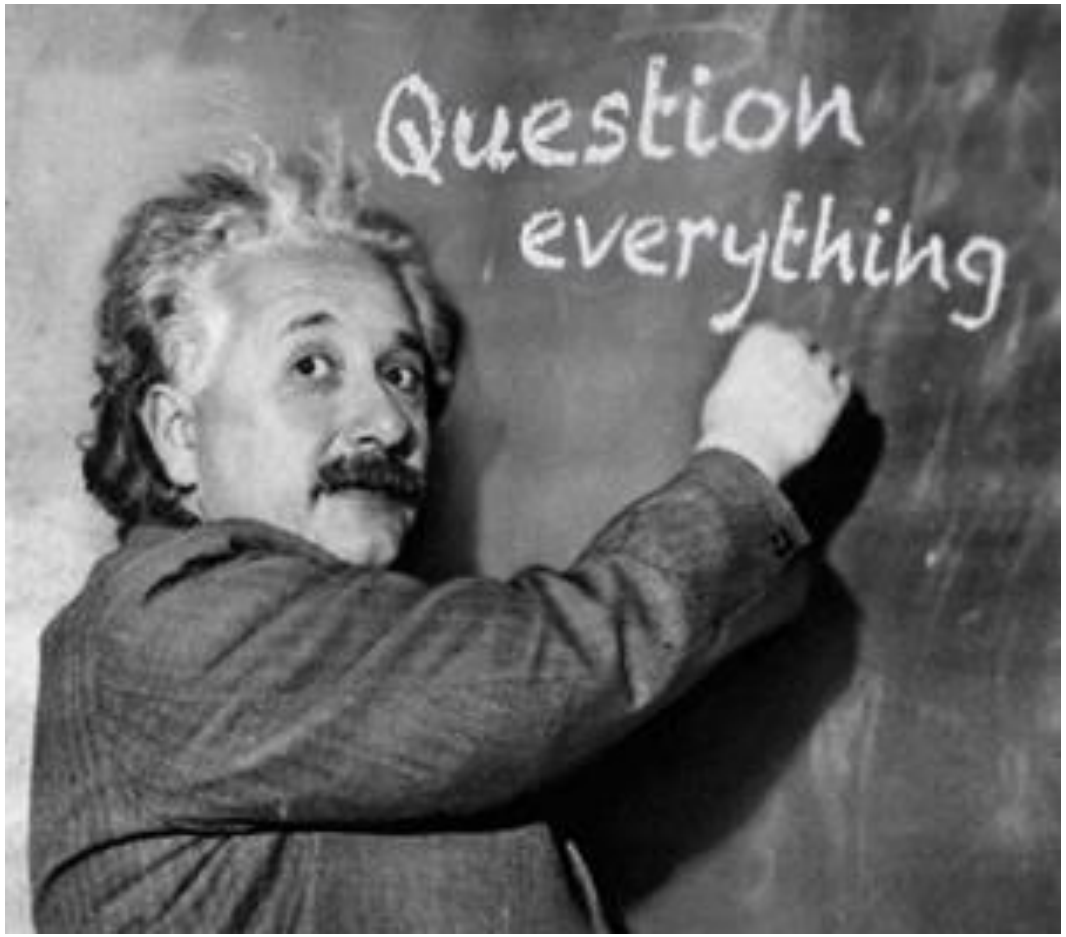
Summary and Conclusions

Most promising metallic NPs proposed for radiotherapy

- Gold (Au, Z = 79)
- Platinum (Pt, Z = 78)
- Hafnium (Hf, Z = 72)
- Gadolinium (Gd, Z = 64)
- Silver (Ag, Z = 47)
- Iron (Fe, Z = 26)

Thank you very much for your attention!!

Questions?



Comments?